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Reagan must live with arms control

Arms control has fallen off the agenda in Washington since the accession of Mr Ronald Reagan on 20 January, and nobody is surprised. Mr Reagan himself has a consistent record of indifference on the subject, now strengthened by the Administration's anxiety to be seen to be tough with the Soviet Union. The new Secretaries of State and of Defense, Mr Alexander Haig and Mr Caspar Weinberger, are unlikely to be urging that the Administration should push for further measures of arms control. In this climate, it is therefore a sign of grace — and of European and especially German influence in Washington — that the bilateral talks with the Soviet Union on the problems of limiting "theatre nuclear weapons" are continuing. Yet arms control cannot remain indefinitely in limbo. Sooner rather than later, perhaps in the last quarter of the year, the Administration will be forced to talk to the Soviet Union about something; European interests that there should be substantial progress in this direction are too strong indefinitely to be ignored. Then the non-nuclear signatories of the Non-Proliferation Treaty who went away grumbling from the review conference last September (and whose silence since is mystifying) will become thorns in the flesh of the superpowers again if nothing is done "to undertake effective measures in the direction of nuclear disarmament" as promised by the treaty. Whatever its inclinations, the Administration will find it has no choice but to talk. Where should it begin?

It should start with domestic housekeeping. As things are, the United States is better prepared for building nuclear weapons than for agreeing to dismantle them. To be sure, there is a dedicated government agency called the Arms Control and Disarmament Agency, which in its time has done sterling service and which has also been a means by which distinguished outsiders could make a mark on United States policy. The trouble is that the agency is only one of several sources of analysis and opinion on arms control proposals licensed to speak their minds in Washington. The State Department has often behaved as if the agency sheltering under its wing was a refuge for hopelessly idealistic liberals. But even the State Department cannot expect to have the last word. The Department of Defense and the Chiefs of Staff are separately entitled to speak up, while the White House itself in normal times is well equipped to disagree with everything that everybody else has said. The result is that proposals for arms control treaties usually find their way to the US Senate encumbered by a bewildering variety of conflicting opinions from within the Administration. Since 20 January, however, the Administration has been even less well prepared for action on arms control. The arms control agency has been without a director, and it is not yet clear whether the proposed nomination of Mr Eugene Rostow, a super-hawk, is intended to enable the agency to stand up to Mr Haig or, alternatively, to emasculate it. (Mr Rostow once told the Senate Foreign Relations Committee that the only agreement between the United States and the "Russians" of which he thoroughly approved was that for the purchase of Alaska.)

Even at this late stage, the Administration would be well advised to rebuild this cumbersome apparatus. The arms control agency owes its existence to the conviction, in the early 1960s, that if arms control were left to the Pentagon and the State Department, nothing would ever be accomplished. It is, however, folly to suppose that the agency could carry through the Senate agreements on arms control to which the Pentagon and the State Department were opposed. Then, with the passage of time, it has

become more and more widely recognized, even by the hawks, that arms control is an integral part of foreign policy and of national security. So much is clear from the working of the healthy clutch of international treaties accumulated since the ban on testing nuclear weapons in the atmosphere signed in 1963. Salt I, which limits the deployment of anti-ballistic missile systems, is more than a mere token of self-restraint by the superpowers. Because of the meetings of the standing consultative commission set up to supervise the working of the treaty, it has become a valuable means of exchanging data and opinions on technical issues. This, for example, is how the function of the Soviet "Backfire" intermediate bomber has been understood in Western Europe and the United States. So, as things have turned out, the process of arms control would be strengthened if the arms control agency were now firmly integrated with the State Department, not left on a limb where it can be disowned whenever the State Department and the Pentagon think it has gone too far. The overriding need is that the Administration should have a mechanism for arriving at one view, not half a dozen, of what it seeks from negotiations.

The Administration must also decide what it is prepared to talk about. The conventional wisdom is that Salt II, the agreement limiting the numbers of Soviet and American strategic weapons that was signed nearly two years ago but never ratified, is now dead. The Soviet occupation of Afghanistan was the occasion (in an election year) for President Carter tactfully not to press the treaty on an unwilling Senate. But nobody is sure of the status of a treaty which is signed but not ratified. In other fields — the Threshold Test-Ban Treaty, for example, which was signed in 1974 but never ratified — both superpowers behave as if the treaty had the force of international law, and complain if they think that one or other of them has violated the agreement. The chances are that both the United States and Soviet governments will find it prudent to behave as if Salt II were a reality. And long before 1985, when the unratified treaty would normally expire, those now saying that Salt II is dead will be crying "Long live Salt III". They will be referring to an amalgam of Salt II and whatever agreement emerges from the talks now under way on the technically daunting problem of limiting nuclear delivery systems of less than strategic range — cruise missiles, Pershing II and, for that matter, the "Backfire" bomber. Salt II is in many ways an unsatisfactory treaty (that could be improved by further negotiation) but the risks of letting it lapse will, long before 1985, seem too great for any administration to contemplate lightly.

The other dead duck from last year's agenda is the negotiation of the Comprehensive Test-Ban Treaty. A year ago, the United States Administration was saying that two months hard talking, and a sufficient measure of political will, would see the treaty signed and sealed. Since the arrival of Mr Reagan and his colleagues, that cheerful talk has quite evaporated. Instead, the military and the weapons laboratories have become eloquent about the risks of dispensing with weapons tests. The problem they identify is real enough, and comes in three parts. First, there are problems of reliability. Nuclear warheads deteriorate in performance with the passage of time, some of them because components such as tritium have relatively short half-lives, some of them because more conventional chemical components go off. The result is that some types of warheads must be refabricated at regular intervals and, in an ideal military world, tested. But there is no reason why the rebuilt bombs should not follow the original designs exactly. Second, there are problems of development. If



somebody designs a new delivery system, should not be weapons laboratories be asked to tailor the ideal warhead to it? The weapons laboratories answer enthusiastically and affirmatively. The truth, however, is that both the superpowers must now have such a range of efficient warheads that the search for perfection cannot substantially affect national security. Third, more important but less tangible, the military (on each side) would be psychologically uneasy (and the weapons laboratories would lose some of their best people) if testing were prevented by means of a comprehensive test-ban treaty. The strength of this objection was consistently underestimated in President Carter's unrealistic times. The result is that too little has been done, in the United States and elsewhere, to prepare the military for living without tests. It would be too much to hope that Mr Weinberger will take on this task so early in his career at the Pentagon. The best hope now is that the pressure of events will persuade him or his colleagues of the importance of the exercise.

For the immediate future, then, the prospect for substantial progress in arms control is far from bright. The new Administration will find itself dragged reluctantly towards Salt III. The Comprehensive Test-Ban Treaty will hang fire until opinion changes towards the second half of Mr Reagan's term. Wrongly, the Reagan Administration will be pilloried when it sets

about the dismantling of President Carter's clumsy anti-proliferation apparatus and may even be persuaded as a result that it had better stay clear of anything to do with arms control. That would be a great misfortune, especially because the agenda the Administration has inherited includes two treaties whose ratification by the United States Senate would bring benefits that are totally unalloyed. The Threshold Test-Ban Treaty (limiting tests to the equivalent of 150,000 tonnes of TNT of explosive power) includes provision for the definition of allowed testing sites, and for the pre-arranged calibration of the seismic characteristics of the underlying rocks, that would immediately improve the efficiency of the seismic detection networks on both sides. The treaty to regulate the use of peaceful nuclear explosions (with which even the Soviet Union now seems disenchanted) provides for similarly open exchanges of technical data about the characteristics of nuclear explosions. Would it not make sense, even for an administration anxious to demonstrate its firmness with the Soviet Union, to ratify (with the assistance of the Senate) these treaties? For as things are, the United States government (like that of the Soviet Union) dares not break the rules agreed, but cannot enjoy the benefits that would accrue from ratifying the treaties. Even Mr Reagan and his colleagues must know that that is absurd.

Can British education be reformed?

The British educational system, long and widely known for its waywardness (the encouragement of school students to abandon those studies of which they are most in need) and excesses (the preoccupation with examinations), may be about to change. The government has now published a document, *The School Curriculum*, that sets out a recipe for a uniform and rational curriculum for British schools and which also promises (some teachers say "threatens") a continuing interest in progress towards its modest goals. Others than the British may not fully appreciate the momentous and historic significance of what the British government has done. Although the education of the young is financed largely from taxation, the central government has no constitutional right to intervene in what is taught in British schools. The only legislative constraint on the school curriculum, embodied in the Education Act of 1944, is that publicly supported schools must provide religious instruction of some kind. For the rest, local educational authorities are constitutionally the final arbiters of the curriculum, but it has never been finally decided whether even they can tell head teachers how the school curriculum should be designed. Indeed, head teachers themselves are frequently at loggerheads with teachers and teachers' unions because their power to decide what should be taught in their schools is circumscribed by teachers' insistence on "academic freedom". Throughout the 1960s, when schools were sufficiently well supplied with funds to be able to experiment with the curriculum, successive central governments justified their cowardly detachment from what has happened by saying that they had no authority to intervene. It is therefore all the more creditable that central government should have chosen to intervene at this point, when there are no substantial funds with which to grease the palms of recalcitrant education authorities.

It remains to be seen how effective the new interventionist regime will be. *The School Curriculum* has only one important argument — that there should be a core curriculum in all publicly supported schools, consisting of language (usually English language), mathematics and science, and that no student in a secondary school should be allowed (let alone encouraged) to give up such a study before the age of 16. In addition, the document says, students in secondary schools "should undertake some study of the humanities designed to yield lasting benefit". The document recites predictable platitudes about the importance of moral education, preparation for adulthood and the like, and is intelligent but also defeatist about foreign language learning. Must not such a moderate and modest programme of reform

surely succeed?

Unfortunately, the outcome is by no means assured. Schools and the teachers' unions have been quick to say that the government cannot will the ends without providing the means — and that there is not merely a shortage of money but of people. For decades, the British educational system has been crippled by the lack of confident teachers of the essential parts of the curriculum as now defined — science, mathematics and modern languages. Even in first language teaching, trendily dubbed "communication skills" by too many schools, the efforts of able teachers are too often offset by those of teachers who believe that any string of words is a measure of a student's creativity, and therefore to be valued. The British government must know that even its modest proposals will not have the influence expected of them unless it can arrange for a general improvement in the quality of teaching. But recruiting more specialists, people with good degrees in mathematics, physics or even French, is no solution. A degree in mathematics is no assurance that a person will be able to explain the working of a slide-rule to a class of 12-year-olds. Nor will similar qualifications in other fields help primary school teachers to persuade even younger children that they are genuinely teachers.

Having embarked on the courageous course of pretending to have an opinion on the school curriculum, the British government has in the process saddled itself with an administrative problem. How will it enable the schools, at least some of which are eager to follow the instructions now implicitly handed down, to do what is asked of them? The most immediate need is not (as the teachers' unions and many schools have been saying) to increase the supply of specialist teachers but to rid the schools system of the tyranny of the public examinations that now dominate the curriculum. Fortunately, having agreed that there should be a reorganization of the public examinations provided for 16-year-olds, the government is well placed to do just this. The ideal would be that it should let schools (or their education authorities) provide school-leavers with suitable certificates and that the public examinations for 18-year-olds, the most distorting of present influences on the school curriculum because of their use as means of selecting students for higher education, should be abolished. Then, paradoxically, the government would find that, having made its point about the pattern of education, it could confidently allow schools to get on with the only task for which they are equipped — teaching young people. Left to themselves, but with clear objectives, they would find the teachers that they need.

European biotechnology lies in disarray

Commission's plan lacks friends

Trouble looms again for the ill-fated European research programme in biomolecular engineering, conceived in 1976 and, after much consultation, proposed to Community states in final form by the European Commission last summer. The Council of Ministers, which has the final authority over such proposals, placed the £16 million (26 million European Units of account (EUA)) five-year programme with its research committee for comment. And the committee now says that it cannot agree on the content, scale or cost of the programme.

The proposal was innovative in its time — it was praised by Jacques Monod and has been broadly backed by European industry, but it now looks unlikely to get approval in any form before November, the next date for a Council of Community Research Ministers. If the research committee had agreed, there was a chance that the programme would have gone through as a "point A" — a proposal that can be approved by any council without debate.

The programme deals with potential scientific bottlenecks in enzyme and recombinant DNA technology — such as provision of cofactors in enzyme-induced synthesis, or problems of expression efficiency in genetically engineered bacteria — through "indirect actions", where the Commission selects and organizes groups around Europe to investigate specific questions and pays half the costs (see *Nature* 283, 125; 1980).

Originally the programme contained six projects, but discussions last year within CREST, a committee of government representatives in which the Commission takes soundings of national views, reduced the number to four, eliminating areas with likely immediate medical or industrial application, and trimmed the budget to 15 million EUA. French representatives requested that half this sum be spent on education and training.

Eventually a "CREST compromise" was worked out, with 4 projects and 15 million EUA of which 20 per cent would be for education. But, at the level of the research committee of council, this compromise has proved unacceptable — as has a further compromise proposed by the secretary of the committee which would reduce the spending still further to 11.8 million EUA. The French members want their 50 per cent education and German members want the number of research projects cut to two, restricting the programme to agricultural applications (basically gene transfer in plants) and

safety questions. Britain appears to back the CREST compromise, or something close to it; all other states would have preferred the original proposal but are prepared to accept that of CREST.

In fact the proposal has fallen foul of the two major obstacles to Brussels-initiated research. First, the concept of a "European" industry benefiting the Community as a whole has not yet taken root, and if a piece of research has a potentially large economic benefit — such as research into cloning vectors, one of the original six proposals — nations are unwilling to share their expertise. The French view of the biomolecular engineering programme exemplifies this attitude: the government wishes the programme to be reduced to a training programme in

recombinant DNA techniques (it has had trouble recruiting for its genetic engineering company, Transgene) and effectively rejects the idea of joint research.

The second objection to Brussels-based work is that if nations already have a research programme in a particular area, they are unlikely to set aside funds in that area for work undertaken outside their control. This is broadly the position of German biotechnologists: they have a large national programme, but research money is tight, and contributing to a Commission-based programme would only distort their priorities.

More Community-minded biotechnologists in both France and Germany, however, point out that long-term progress

German physics in row about Einstein

The German Physical Society (Deutsche Physikalische Gesellschaft) has been shaken by a fierce row over an attack on Einstein published last November in *Physikalische Blätter*, the society's membership journal. Now the editorial board of the society, and the president of the society, Professor Horst Rollnik of the University of Bonn, have dissociated themselves from the original article, by Professor Albrecht Unsöld of the University of Kiel. In a statement in the March issue of *Physikalische Blätter*, they say that Unsöld's article contains statements that are open to misinterpretation, or even false, and promise that the journal will carry an article by an historian of science dealing with Einstein's links with Germany. There is, however, no suggestion that the journal will publish the critical letters it has received in the past few months.

Unsöld, a theoretician now 75, appears to have begun his attack on Einstein at a symposium held at the University of last May, when he said that Einstein (and Haber) was guilty of crimes "no less serious than those of Hitler".

Unsöld says that Einstein's earliest papers were deficient in references to the work of other physicists because of his "narcissism". Unsöld says that Einstein had run out of new ideas by the age of 45, for which reason he turned his attention to public affairs and in particular to "Jewish nationalism", put on a par with the attachment of other physicists (Lenard, Stark and Wien, for example) to German nationalism. The article says that Einstein's move to Berlin in 1914 was motivated by his "enormous salary demands", and that he was frustrated at not being awarded a Nobel Prize sooner because he needed the prize "in order to divorce his first wife".

The article also says that Einstein

travelled outside Germany with increasing frequency after 1920 "to fill the emptiness created by the drying up of his more profound ideas" and to "make propaganda" for the theory of relativity and for the creation of the state of Israel. Unsöld says that Einstein should have foreseen that his letter to President Roosevelt in 1939, confirming the opinion that nuclear weapons could be built, would lead to the destruction of Hiroshima and Nagasaki. He goes on to urge sympathy for those physicists who stayed behind in Germany after the arrival of Hitler, and who were able to resist the penetration of university faculties by "party personalities" while safeguarding the traditions of research and the university libraries.

There has been a torrent of protests against the publication of Unsöld's article, especially from German physicists working abroad. Unsöld has been charged with antisemitism and with mischievous inaccuracy. In reply to one of these complaints, Professor Rollnik explained that he had not seen the article before publication, that its appearance could be accounted for only by a "gross failure" of coordination between the editorial board of the journal (appointed only in 1980) and the editorial staff and that new rules have been devised to prevent the repetition of "such a nasty event".

The disclaimer now published in the March issue of *Physikalische Blätter* says that the journal must be free to publish controversial opinions even when most members of the society disagree, but that Unsöld's article is inaccurate and that its author has only harmed himself in his attempt to defend physicists who worked in Germany during the Third Reich. It remains to be seen whether it will suffice to bring the row to an end.

on applied biology — to which the Brussels programme is directed — depends on the scale and quality of communications among scientists, and that the principal contribution of the biomolecular engineering programme would have been to set up a network of working contacts throughout Europe. A single European state does not have sufficient biotechnologists to make significant advances in competition with the United States or Japan, they argue.

This point appears to have been lost on the research committee of the Council of Ministers, which has now passed the problem to the diplomatic level in Brussels — the committee of permanent representatives, Coreper. But with the present low level of diplomatic concord in the Community, there seems little hope of a resolution.

● Britain's recent white paper on biotechnology was roundly condemned by British researchers at the second European conference on biotechnology at Eastbourne last week. Dr Duncan Davies, whose chief scientist's office at the Department of Industry produced the white paper, made a flying visit to the meeting to defend his position. He was subjected to a barrage of speeches from the floor, but managed to squeeze in that he would review the white paper's provisions on the education and training of biologists. Informed opinion, however, expects little to come of such a review. **Robert Walgate**

Taking note, or shelving?

The British House of Commons last week "took note" of the European proposed programme on biomolecular engineering, a mechanism which allowed the Under Secretary of State for Industry, Mr Michael Marshall, to state the government position on the latest version of the programme.

The original proposal (for 26 million European Units of Account (EUA) over five years) was "over-ambitious in both scope and expenditure", said Mr Marshall; but the shift of emphasis away from research and towards training in the revised proposals (an 11.8 million EUA four-year compromise proposed in Brussels) "is of an extent not hitherto contemplated".

Without naming France, Mr Marshall indicated that the government did not share the French view that there is a significant lack of training opportunities. Nevertheless Britain would continue to work towards an acceptable compromise, he said.

Tam Dalyell MP, described by one member of the House as "almost a walking Select Committee on Science and Technology", warned the minister to be wary of the French position, saying that the new proposals "by pooling training, hand over British expertise to the French for little or no return". The House took note. **Robert Walgate**

European Space Agency Space on the cheap

Paris

The member states of the European Space Agency are still at odds about the future. Although they have agreed to the proposal of Mr Erik Quistgaard, the agency's director-general, that the budget should be reduced to 60 per cent of its present level over the next ten years, they are hopelessly divided on how to spend the money. The problem is that the reduced budget will not support the three programmes that have been put forward — in telecommunications, space transportation systems and science. So the agency is wrestling with priorities.

Quistgaard's suggestion that the mandatory science budget, to which all member states must contribute, should be increased by 60 per cent over the next ten years has so far fallen on stony ground. Britain, Sweden, Spain and Belgium have flatly turned down the proposal; only Germany has agreed to contribute in full.

Lacking unanimous agreement, the agency may therefore have to take the unprecedented step of admitting optional contributions to scientific programmes. That, according to Ernst Trendelenberg, director of scientific programmes, could divide European space scientists into privileged and under-privileged depending on their nationality.

Germany and France are the most likely to favour this approach, but for different reasons. Germany would like to divert some of the money spent on Spacelab to science, and France is keen that there should be a steady stream of satellites for launching with Ariane, whose development it has supported heavily. Britain, on the other hand, says the agency is too inefficient to justify more spending on science.

The most heated debate in the agency, however, is about the choice of space applications for the next ten years. The two chief candidates — space transportation systems and telecommunications — have been proposed by France and Britain. France argues that Europe must have a launcher to rival the space shuttle, especially if materials processing experiments on Spacelab demonstrate that space has commercial potential. It would like to develop more sophisticated versions of Ariane and ultimately an unmanned partly-reusable space transportation system called Solaris, which is expected to be put before the agency's council at the end of the year.

France disagrees with Britain's proposal to spend more on telecommunications on the grounds that that should be left to industry. Both France and Germany withdrew from early plans to build a large satellite for direct broadcasting, preferring instead a bilateral programme. Neither believes that the "Large Satellite Programme" (L-sat), due to be approved

Quistgaard awaits orders

Paris

Erik Quistgaard, director-general of the European Space Agency, is optimistic. The present stalemate between the member states, he says, might be broken if the users of space technology were more intimately involved. Thus post offices, users of remote sensing data and industrial users of experiments on Spacelab rather than government departments should have more influence over what the agency does. The problem is that although most states know what they want from space, few are decided about their expectations of the agency.

Mr Quistgaard believes that it is up to member states to tell him what they want. Some delegates, however, are looking to him for solutions. The rejection of his proposal that the science budget should be increased is hardly encouraging.

Quistgaard believes that Europe can and should compete with the United States and others in telecommunications, remote sensing and space transportation systems. He favours unmanned space transportation systems as an alternative to the space shuttle. **Judy Redfearn**

in June by Britain, Italy and some of the smaller countries, goes sufficiently beyond their own plans to justify participation. Britain, on the other hand, with a smaller space budget than either France or Germany, sees L-sat as its opportunity to compete for world markets.

The hope is that these two conflicting views can be reconciled. Professor Hubert Curien, director of the Centre National d'Etudes Spatiales, believes that Britain may be moving towards the view that space transportation systems must be developed within the agency. And John Hawkes, of the British Department of Industry, thinks that his French colleagues may now be more ready to agree that telecommunications development beyond L-sat should be supported on a European scale.

If Mr Quistgaard is to put his ten-year plan into operation, he needs to know soon which of these options the agency should be studying. To pursue both would entail scrapping the agreement reached on the future budget. **Judy Redfearn**

Cystic fibrosis

Diagnostic hopes

Techniques now being developed at various centres in Britain and the United States have stimulated optimism about new approaches to the prevention of cystic fibrosis. Workers in the field are particularly excited by the prospect of being able to detect carriers of the genetic defect.

Among genetic deficiency diseases, cystic fibrosis is peculiar in that the nature of the underlying molecular defect is as yet unknown. The belief that only a single gene

is involved has, however, recently been confirmed by Romeo (Bologna), working with records of dispensations for cousin-cousin marriages accumulated at the Vatican. Among Caucasian populations, the frequency of carriers of defective genes is thought to be 1 in 20. The Vatican records have confirmed that cystic fibrosis is an autosomal recessive disease, accounting for the normal incidence in Northern Europe of 1 in 1,600.

Attempts at antenatal diagnosis of cystic fibrosis have been based on the virtual absence, in those with the overt disease, of an uncharacterized proteolytic enzyme. This technique, originally developed by Dr Henry Nadler in Chicago, is now being taken up at other centres. It may eventually be possible to offer amniocentesis to pregnant women who have already produced one child with cystic fibrosis, although the frequency of false positives and false negatives has not yet been determined confidently.

Two techniques for the recognition of carriers are being developed, one of which is based on the occurrence in the serum of both homozygotes and carriers of a protein apparently characteristic of cystic fibrosis. Last year, Dr David Brock's group at the Department of Human Genetics at the University of Edinburgh reported the raising of antibodies against the protein (Manson, Jean and Brock, David J.H., *The Lancet*, 16 February 1980) and the possibility that these might be used to distinguish between cystic fibrosis patients (carrying a full dose of the protein), carriers (with a smaller amount) and normal people. Since then, better techniques for raising antibodies have been developed, and there are plans for the development of monoclonal antibodies. The second technique, developed in Los Angeles, is based on the differential response of cystic fibrosis patients, carriers and normal people to the administration of the drug ouabain.

The hope now is that a relatively straightforward way of recognizing carriers may reduce to manageable proportions the provision of amniocentesis for mothers at risk of producing children with cystic fibrosis. In Britain, the Department of Health is under some pressure to prepare for a pilot scheme against the time when the diagnostic techniques are fully developed.

Genetic screening may well be possible before the nature of the underlying defect is understood. One possibility is that the protein characteristic of cystic fibrosis is in normal people removed by the proteolytic enzyme which is the basis of proposed diagnosis of the disease by amniocentesis. In Britain, Professor R. Williamson (St Mary's Hospital Medical School) has embarked on a programme for the identification of the cystic fibrosis gene using techniques of chromosome sorting and genetic manipulation.

Financial support for most of the British research in this field is provided by the

Cystic Fibrosis Research Trust, which is now spending more than £500,000 a year. It is a sore point with those involved that the Medical Research Council's contribution to work in the field, although small, was exaggerated in a reply to a parliamentary question last October.

Safety at Windscale

Yes we were wrong

The British nuclear industry's site at Windscale, the source of a long sequence of mishaps in the past decade, was last week given an adverse report by the Health and Safety Executive, the organization in charge of the Nuclear Installations Inspectorate. And British Nuclear Fuels Limited, the owner and operator of the site at Windscale, put its hand on its heart and acknowledged that it had been at fault.

The report by the Health and Safety Executive is largely concerned with managerial failures at Windscale. It says that British Nuclear Fuels had allowed the condition of the several plants at Windscale to deteriorate until by the early 1970s, their safety could not be assured; and that the efforts made since 1974 to enhance the safety of the Windscale facilities further complicated the assurance of safety by their demand on resources. The report complains that the company paid too little attention to the filling of senior posts at Windscale and that the arrangement whereby the Northwest Area General Manager is responsible for safety in the whole of Windscale but also at other plants means that his responsibilities are too much diluted.

The report says that most incidents at Windscale have arisen because of mistakes in the execution of routine tasks. About a quarter of the 30 or so incidents reported each year since 1976 have involved the exposure of people to doses of radiation exceeding the limits laid down. The inspectors say that the managers of the plant must provide workers on the site with regularly updated and more explicit instructions for carrying out routine tasks and that a promised review of safety procedures should be completed more quickly than now seems likely. In the long run, the Nuclear Installations Inspectorate wants to see a system for the independent audit of safety at Windscale.

The effect of the Health and Safety Executive's report on the publicly owned parts of the British nuclear enterprise is likely to be far-reaching. British nuclear engineers appear to acknowledge that Windscale, because of the way in which it has been created by the addition of new plants to an already overcrowded site, and because of its relative antiquity, is not the ideal model for the safe operation of nuclear plants. But other nuclear sites are likely to encounter similar problems which will be less readily excused.

US university regulations

Complaints listed

Washington

Jumping on the anti-regulation bandwagon which helped propel Mr Ronald Reagan into the White House last November, US colleges and universities are eagerly compiling a list of federal regulations which they feel are stifling the productivity of their educational and research efforts.

One regulation that the universities have in mind is the notorious Circular A-21, issued by the Office of Management and Budget, laying down strict accounting rules which must be obeyed by any scientist and his or her institution receiving federal research support. "A-21 is at the top of our priority list for reform", Mr Sheldon Steinbach, general counsel of the American Council on Education, said last week.

Another rule which the universities would like to see changed is the requirement that scientists should provide a precise account of the way that their time is divided, not only between teaching and research, but also between different research projects. Another relates to cost-sharing between universities. And although the Carter Administration, in its last month in office, took steps through the Office of Management and Budget and the Office of Science and Technology Policy to reduce the impact of the A-21 rules, many universities feel that they are still far from satisfactory.

The universities' point of view had already been presented to Vice-President George Bush, who has been appointed by President Reagan to head a task force on regulatory reform, and has announced the Administration's intention to defer several new regulations proposed by Mr Carter, and also to take a close look at several existing rules to see if they should be revised.

Last week, Mr Bush was presented with a nine-point plan to streamline federal rule-making which had been drafted by 62 leading businessmen and educators organized by the American Council on Education into a Business Higher Education Forum. The forum's statement linked together the complaints made by the business community about, for example, the impact of health and safety regulations, and those coming from the academic community about the general burdens of educational regulation.

The report of the forum says that, as a result of the many educational reforms introduced over the past twenty years, from rules about facilities for handicapped students to affirmative action programmes, regulation of higher education "has mushroomed", with 400 laws on the statute book overseen by 34 committees of Congress.

One complaint is the economic impact of the regulations. According to Howard Bowen, an economics professor at the

Claremont Graduate School, federal regulations alone inflate costs on 3,200 US campuses by an estimated \$3,000 million a year. And the Business Higher Education Forum quotes the widely used — although often challenged — calculation by economist Murray Weidenbaum, now chairman of the Council of Economic Advisors, that federal regulations may be costing US industry \$100,000 million a year overall.

But there are other factors at work as well. "The most important of the regulations' effects is the growing and unwelcome degree to which government intrudes into the private sector, compromising the capacity for independent action in business, labour, academic institutions and elsewhere", says the forum report.

The Bush task force has already said it plans to review two existing regulations covering research. One requires college professors and researchers receiving federal grants or contracts to obtain government approval for leave of absence, changes in research methods or transfer of the research to others; the other requires colleges and universities to share certain percentages of the cost of research financed by the federal government.

Research administrators are cautiously optimistic that, given the general anti-regulatory stance of the new Administration, they will achieve significant changes, a step towards what the American Council on Education forum describes as "a more systematic, rational national policy on regulation".

David Dickson

French nuclear power

Close to the brink?

Brussels

The breakdown in the electricity supply from the French national grid to the nuclear reprocessing plant at Cap de la Hague, at the tip of the Cherbourg peninsula, which occurred in April last year (see *Nature* 24 April 1980, p. 653), came within an ace of becoming a major catastrophe. That, at any rate, is what the Brussels-based international socialist group Agenor is claiming.

Agenor has published an account of the incident based on the research of two groups of critical scientists, GSIEN in France, and PERG (Political Ecology Research Group) based in Oxford. The report claims that when the electricity supply was restored the emergency generator "blew", putting all the plant's electrically operated mechanisms out of action. By pure chance one of the worst consequences was avoided. Ten minutes earlier a 30-kg load of plutonium was being prepared. Without the electricity, the agitation needed to prevent the plutonium going critical would have stopped.

The power failure also stopped the cooling of the reservoirs containing radioactive waste from all of France's reactors.

Nuclear Structure Facility

More delays ahead

Britain's Nuclear Structure Facility, under construction at the Science Research Council's Daresbury Laboratory in Cheshire, has run into new problems. The 30-MV tandem Van de Graaf accelerator had been expected to produce its first beam for experiments this month, but the latest setback means that full operation is unlikely before the autumn. The problem is dust, which caused a short circuit across the laddertron — the aluminium and plastic belt which carries charge up the 40-m column to terminals — during a recent trial. The resulting break in a couple of plastic links in the laddertron has been relatively simple to repair, but getting rid of the dust is proving more difficult.

The Nuclear Structure Facility, which should provide a variety of ion beams for nuclear structure studies, has had a long, and not always easy, history. The Science Research Council bit off rather more than it could chew when it decided in 1972 to build a tandem Van de Graaf accelerator that exceeded the capabilities of machines operating at the time. The highest voltage achieved by standard machines was then about 15 MV. Design and technical problems have considerably increased the

Within 10 hours the waste would have begun to boil, releasing caesium-137 and rubidium-106 and making it difficult to gain access to the plant to restore the current and restart the cooling. If that had happened, within three days a radioactive cloud would have formed, and the prevailing southwesterly winds might have carried the cloud as far as London. The uncooled and dry waste left in the reservoir would then have melted down, reaching the water table and causing a steam explosion. At the Cap de la Hague site not only radioactive waste but also stored plutonium would have been distributed over a large area.

On 6 January this year a different accident occurred, the news of which the authorities had more difficulty in suppressing. A fire broke out in a trench used for dumping cladding removed from around the used fuel rods of graphite gas reactors. When alarm bells rang in several workshops on the site, the reaction was to disconnect them. Only later was it realized that radioactivity was coming into the workshops from outside. The fire burned for 15 hours. According to measurements taken by the unions, the level of beta radiation on the site had risen to 26 times the permitted level and alpha radiation to 6 times the permitted level. According to Agenor, the French Atomic Energy Authority (COGEMA) avoided informing the workers downwind of the fire and denied there was any danger to the local population. The radical trade union CFDT had produced figures to show that milk has been contaminated throughout the

building time and the cost.

The dust problem first became apparent last November. There are two sources of dust — beds of alumina pellets used to dry out sulphur hexafluoride gas which surrounds the tandem, and the bearings used in the construction of the machine. A short test last summer revealed no dust problem. For that test, the machine was run without drying the sulphur hexafluoride insulating gas and, according to Dr R. Voss at Daresbury, not for long enough for the dust from the bearings to become a problem.

Work is now concentrated on removing all dust from the machine. Dust from the alumina beds should be minimized by passing the gas through more slowly, but it may be more difficult to eliminate dust from the bearings. However, after a few hundred hours of operation, bearing dust should fall off dramatically. After that, the plan is to catch residual dust in traps built into each rung of the laddertron.

The result is that the facility will be late coming on line. It is now hoped that the high-voltage performance can be demonstrated within one or two months, the accelerator tube within the following two months and that commissioning will be complete two months after that. The first experiments are likely in September or October.

Judy Redfearn

Cherbourg peninsula.

The intention of GSIEN, PERG and Agenor is to discredit the technology of the Cap de la Hague reprocessing plant and to go on to show that the nuclear power strategy of France as well as Western Europe is wrong.

As well as having problems with safety, the Cap de la Hague plant is not fulfilling its tasks. Cap de la Hague started processing oxide fuel in September 1976, a year behind schedule. The planned capacity is 800 tonnes a year but by 1980 the total amount processed was only 340 tonnes. In the meantime, France has gone on negotiating contracts to reprocess the waste from Japan, Germany, Belgium, Spain, Sweden, the Netherlands and elsewhere. To manage what is estimated to be a commitment to reprocess 6,000 tonnes, France is building two more plants, each with an annual capacity of 800 tonnes. Neither is likely to be in operation by 1988, and meanwhile the waste is being stored.

As well as being behind in coping with nuclear waste, Cap de la Hague is not producing enough fuel. In 1980 the plant handled enough uranium oxide to produce only one tonne of plutonium. Agenor argues that at this speed the fast-breeder strategy falls flat on its face. The initial fuel load of plutonium needed at Malville, a 1,300-MW reactor, is 4.5 tonnes, so that the idea that France or Europe can become self-sufficient with fast-breeders is implausible. Europe boasts only one other reprocessing plant, that at Windscale in the United Kingdom.

Jasper Becker

CORRESPONDENCE

Genetic weaponry?

SIR — The problems of implementing the Helsinki accords in Soviet Russia have been raised once more in your issue of 15 February (p.437) where the case of David Goldfarb was mentioned. I have known David Goldfarb for many years. He was head of the Laboratory of Molecular Genetics of Bacteria and Bacteriophage of the Soviet Academy of Sciences. He has asked to emigrate to Israel and a visa was denied on the grounds that "he had access to classified work".

David Goldfarb was never involved in classified work and did not even have security clearance — as so many scientists in Soviet Russia have. He worked on the genetics of bacteria and bacteriophage. If such research is classified, it means that Soviet Russia intends to use molecular genetics for biological warfare.

So far as is known, the USSR Academy of Sciences is considered when a scientist asks for an emigration visa. One wonders if the Academy will officially and openly subscribe to the decision of the Soviet authorities according to which molecular genetics is a potential weapon and a military secret.

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NRPB and risk

SIR — It must be admitted that one does not know what happens within an organization without being a part of it. It is equally true that being a part does not automatically make one a reliable critic of it. We have these assertions in mind upon reading Mr S. G. Goss's letter on risks at the National Radiological Protection Board (*Nature* 289, 316; 1980).

We have the highest respect for the scientific integrity of Sir Edward Pochin and the late Dr G. W. Dolphin, and their thoughtful attempts to publish reliable information on radiation risks.

We further believe that this view is shared by other US colleagues who have had the opportunity of interacting with these two. Derogatory evidence would have to have a decidedly firmer foundation than that offered in the Goss letter to change this viewpoint.

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Conservation sites

SIR — In his letter of 12 March 1981, Dr C. Muir criticises actions and advice of the Nature Conservancy Council (NCC) over Sites of Special Scientific Interest (SSSI) on the grounds that such sites are misguidedly chosen to protect endangered species, because species extinction is a natural and inevitable process; and that the site notification procedure imposes an unacceptable financial burden on landowners. The NCC does not notify SSSIs from "caprice" but because Parliament in its wisdom conferred this duty upon us, as a key part of a national strategy for nature conservation. We continue to do this because a very large number of concerned people believe that SSSIs are important to the conservation of wildlife and physical features.

Most extinctions during the last 2,000 years have been man-induced, and it is this *accelerated* loss that we are trying to stem. By

2000 AD the world will have lost no fewer than one million of the plant and animal species existing today, mainly through habitat destruction and over-exploitation. In Britain many indigenous species could become extinct. Whether this is held to be evolution or not is irrelevant: the fact is that many people do not wish such losses to occur and look to NCC and other bodies to prevent them.

SSSIs are in any case not chosen just for the presence of rare or endangered species, but to give a national network of important semi-natural habitats. Many are selected quite separately for the importance of their geological and physiographic features. The term "scientific interest" is interpreted broadly as the value of the biological and physical attributes of a site to that informed sector of society which has a concern for such phenomena. The NCC has defined criteria for evaluation of these attributes based on the full range of public interest involved, which is clearly wider than scientists alone.

The SSSI device is one of the few means available for defending the important areas for nature conservation but its actual inadequacy in this respect is currently the subject of lengthy parliamentary debate on the Wildlife and Countryside Bill. Our concern is indeed to safeguard these sites in perpetuity. It is a staggeringly narrow and mechanistic view which holds that once "objects or phenomena" have been "studied" they are of no further value to science and can, by implication, be allowed to disappear. And beyond this, who has the confidence to assert that all possible knowledge has been gleaned from any object or phenomenon?

The responsibility of government to cater for nature conservation as a public interest is not, in a democratic society, altered by the fact that some people find the resulting measures inconvenient to their material interests or irrelevant to their personal beliefs. And just to get things in proportion, the sense of official priority accorded to nature conservation in public affairs is reflected in the £10 million annual budget of NCC, compared with £700 million allocated to agricultural support and £9,500 million to defence.

The NCC has long accepted that when owners and occupiers of land are asked to incur a material loss in order to sustain a nature conservation interest, the principle of compensatory payment is entirely fair. This principle would remain an integral part of the additional site safeguard measures which NCC has been seeking in the Wildlife and Countryside Bill. It seems equally reasonable to ask that owners and occupiers of land accept that they have, on behalf of the nation, a responsibility in stewardship for the national heritage of nature which is now suffering such severe and accelerating losses. We are all increasingly enmeshed in a single society, and when the owners and managers of our national resources ask the taxpayer to help their private circumstances by direct or indirect cash hand-outs, another point of principle arises. This is that these parties should also accept that such funds are distributed across the whole wide spectrum of taxpayer interests, according to a balance of adjudication through the proper agencies of government, of which NCC is one.

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Selling Darwin

SIR — So much has been written on Darwin and Marx, since Halstead's original diatribe against the use of cladistics in the displays of the Natural History Museum (*Nature* 288; 208; 1980), that I wonder whether these two venerable gentlemen are not now reaching for their pens to set the record straight. Until *Nature's* Olympian warnings (26 February, p.735) on the "selling out on Darwinism" in the museum (and by association other new evolutionary approaches) one felt safe from the bogies in Halstead's head. Now that they are out and about rather than dead and buried, it is amusing to consider the original error which gave rise to them.

Halstead's polemics "follow like a tedious argument of insidious intent" (T. S. Eliot). His accusation that cladists and proponents of "punctuated equilibria" are furthering the Marxist concept of dialectical materialism reveals his ignorance of the fundamentals of this concept and of Darwinism. If it is necessary to find a biological phenomenon in keeping with dialectical materialism, then Darwin and not "punctuated equilibria" provides a perfect example. According to dialectical materialism (unsullied version) there are two inter-related components to every process in that a gradual accumulation of small *quantitative* changes leads inevitably to a *qualitative* change of state. Engel's favourite scientific example was the boiling of water. A qualitative change from liquid to gas only occurs after a sufficient quantitative increase in the thermal agitation of the molecules has taken place. According to popular Darwinism a gradual quantitative accumulation of allelic differences as an adaptive response to a specific environment leads inevitably to a qualitative change of state (the inception of reproductive isolation: speciation). If dialectical materialism upsets Halstead he would do better to dismantle 130 years of Darwinism.

Ironically the precise mechanism by which natural selection could increase reproductive isolation perplexed Darwin and, despite the subsequent attempts of Fisher, Muller and Dobzhansky to explain how this might happen, we have little empirical data. "Let me first say that no man could have more earnestly wished for the success of Natural Selection in regard to sterility than I did, I always felt sure it would be worked out but always failed in detail." (Darwin: letters to Wallace and Huxley). Notwithstanding these doubts and lack of data Darwinism flourishes today as an explanatory mechanism of adaptation that leads to speciation.

The theory of "punctuated equilibria" does not see species formation necessarily as the inevitable consequence of microevolutionary adaptive differences. It dissociates (to an extent) quantitative (microevolutionary) from qualitative (macroevolutionary) phenomena. Thus with regard to the process of adaptation, it is Darwinian. With regard to the process of speciation, there are several current notions (ranging from possible developmental constraints on phylogenetic progress to possible biological effects of saltatory and rapidly spreading changes in genome organization) that, although novel to a degree, leave no room for instant creationism or the demise of Darwin, in the way it is construed.

They are basically concerned with differential rates of evolutionary change; and the precise role of selection has not, as yet been ascertained. We should not be too surprised if speciation turns out to have an accidental as well as an adaptive component. The imaginative and enquiring spirit of Darwin lives in all except the minds of those with other axes to grind.

I have a feeling that Darwin would be overjoyed at the general acceptability of his concept of fine-grained adaptation under natural selection, and would not be unresponsive to its possible dissociation as a process from that of speciation. Marx, on the other hand, could but grunt that a dissociation of the two processes no longer requires the inevitability of a change of state of one species into another. GABRIEL DOVER

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From the museum...

SIR — As music is the food of love so surely debate is the nourishment of science, but the crashing cymbals of your leading article of 26 February were a discordant accompaniment to a meagre meal¹. Your subsequent article² sets the stage for a more reasoned discussion.

Controversy and debate over the hypotheses associated with the concept of organic evolution have recently received considerable attention. It can hardly be described as a rot permeating just the British Museum (Natural History) but rather the open questioning of evolutionary theory by serious biologists and philosophers — a fact apparently denied in your first leading article. Furthermore there are clear indications of unease amongst educationalists^{3,4} at the way in which the concept of evolution is being taught, not as an hypothesis open to criticism but as a fact.

Many practising biologists and teachers echo Olson's remark "Evolutionary theory should be treated like any other scientific theory, as a matter for dispassionate and objective study of the evidence available"⁵. Yet this only reflects the more pungent and provocative comment made by Darwin's "bulldog" over a century ago "... the scientific spirit is of more value than its products, and irrationally held truths may be more harmful than reasoned errors. Now the essence of the scientific spirit is criticism"⁶.

The views of many scientists in this museum are encapsulated in a quotation from the museum's "recent brochure":

Biologists try to reconstruct the course of evolution from the characteristics of living animals and plants and from fossils, which give a time-scale to the story. If the theory of evolution is true, the features used to classify species in groups ... were acquired by the common ancestor of the group and inherited by the living descendants ... In this light, the groups-within-groups of classification are seen as the descendants of more or less remote common ancestors. And classifying animals and plants is a way of expressing ideas about the course of evolution. This is why classification is interesting and important to biologists, and why the work of classifying is never finished. For new discoveries lead to new ideas about the course of evolution.

Our understanding of the theory of evolution is that it is an amalgam of many subsidiary hypotheses, related not only to the patterns of

organic diversity but also to the mechanisms whereby they arise. Here we are faced with a core theory and its protective satellite belts. Clearly various aspects of these hypotheses have been criticized and improved upon, and there is no reason to believe that this evolution has stopped, nor should we pretend that it has stopped. As practising systematists we recognize and we do not underestimate the enormous heuristic value of the theory as a stimulus to research.

The theory of evolution must surely be considered an "open question" and we therefore agree that "in the public presentation of science, it is proper whenever appropriate to say that disputed matters are in doubt"⁷. We cannot see, however, how the views expressed in the museum's brochure indicate any divergence from such an objective. Rather, they make a positive statement about the utility of the theory as an explanation for the diversity of life.

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SIR — Dr Colin Patterson¹ says that cladistics is "not about evolution". Cladistics may therefore be considered essentially a method of classification. Based on morphological characters it arranges taxa into a hierarchy and the hierarchy itself has no necessary phylogenetic implications². Since cladistic classifications are based on "derived" similarity, it seems to have been accepted³ that they may resolve the classification hierarchy more fully than traditional classifications, which are based on "clustering" similarity⁴.

If this is all there is to cladistics then at least its taxonomic substance seems uncontroversial. However, any such hierarchical classification, whilst practically useful, is essentially non-scientific⁵, as the aim of science is to explain simple taxonomic relations, then to test the explanation (scientific theories) to see if they make sense⁶. Evolutionary theory is one such scientific theory⁷.

Cladistic method can be incorporated into evolutionary theory by simply accepting that the cladistic hierarchy represents an assumed time axis, a view taken by Hennigian cladists. A historical sequence is "predicted" from the morphology of organisms and in context of Popperian concepts of science, cladists have gone on to compare the congruence of such predicted sequences with other such sequences based on different, semi-independent lines of evidence, such as historical geography⁸.

However, stratigraphy can also be used constructively in such comparisons to call into question the sequence of taxa presented by cladograms and thus to test the usefulness of the hypothesis outlined above⁹.

Cladists to date have applied the concept of testing mainly from an internal perspective of character analysis¹⁰, and historical time-space coordinates of taxa have in general been ignored. The cladogram has thus been regarded as absolute, whilst evidence from stratigraphy, because it is incomplete, has been recognized as relative — and apparently therefore as worthless¹¹. Little attempt has

been made to hold the cladistic system itself up to test. It seems to have been overlooked that cladistic classification may make morphological evidence seem convincingly complete, even though it is not actually complete. I do not understand how a system that is relative (cladogram) can be assumed to be absolute and thus used universally to ignore or "refute" historical evidence that is actually true¹³. The view promulgated here regards both sets of evidence as relative, and thus as commensurable. Cladograms present a relative sequence and stratigraphy presents a relative sequence, and both have their own semi-independent corroborators and falsifiers. In actual practice the testing role of stratigraphy would therefore be difficult, a sign of a mature science^{9,12}; in some cases the stratigraphy may be trusted more than the cladogram, in others the cladogram may be relatively well corroborated¹³.

It is, on the other hand, naively easy to question the cladistic system as a basis for delimiting species. Since cladistic hierarchies reflect characters of organisms, and since such characters are expressed as a hierarchy of proper sub-classes (monothetic sets)⁴, a logical consequence is that classes cannot be discriminated from individuals within the context of character analysis. Men and women form cladistically distinct taxa, and pregnant women a taxon nested within women as a whole. Straightforward genealogical observation refutes this, since a pregnant lady can deliver a son and return to her generalized condition. This argument may seem facile but for the fact that one cladist has recently described several new species out of what many evolutionists might take to be sub-specific populations¹⁴. At any rate there is a potential danger of over-splitting taxa.

My view differs from some of my colleagues in that I maintain cladistics is about evolution and that any sensibly testable system will recognize that evolution is a complex, mature theory, requiring complex modes of testing beyond "naive falsificationism". Basically I consider evolution and the fossil record as very much the proper context for cladistics and not the converse. It is surely commonsense to use historical evidence to examine history and not to view it askance through a haze of global aristotelian philosophy of character analysis, as transformed cladists appear now to be doing. Transformed cladists may justly have rediscovered Aristotle, but if they decide to wipe out Galileo they reject not only Darwin but all of modern science¹². CHRISTOPHER HILL

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NEWS AND VIEWS

Tissue-specific expression of α -amylase

from R.A. Flavell

TISSUE-SPECIFIC expression of eukaryotic genes is always intriguing but of particular interest are cases where the expression of a given type of protein is confined to a few tissues, yet where the level of expression in each of these tissues differs. The α -amylase genes belong to such a system. α -Amylase is produced at high levels in salivary glands and the pancreas, but at much lower levels in the liver. Previous studies on the α -amylases of the laboratory mouse have suggested two structural genes, designated *Amy 1* and *Amy 2*. Several recent papers from a group at the Swiss Institute for Experimental Cancer Research describe the molecular organization of these genes. A single gene, *Amy 1^A*, encodes both the liver and salivary gland amylases (which are identical proteins). Differential expression of these two mRNAs probably requires the use of at least two different promoters for a single cellular gene. Equally interesting, differential splicing is required for the generation of these two overlapping, but non-identical mRNAs. The second gene (*Amy 2*) encodes pancreatic α -amylase, which has a different primary sequence from the salivary gland/liver forms (Schibler *et al. J. molec. Biol.* **142**, 93; 1980; Hagenbüchle *et al. Cell* **21**, 179; 1980).

The notion that differential splicing can be used to regulate gene expression is not new. Indeed, this principle was borne out by the detailed study of viral transcripts where, in several cases, overlapping genes have been found. A well known example is that of the gene products of the early region of simian virus 40, the tumour antigens T and t. t is encoded in a DNA segment colinear with the T gene. Although t mRNA is spliced, this splice occurs after the stop codon and does not, therefore, affect the t antigen protein-coding regions. T antigen is produced from essentially the same mRNA by splicing the region encoding the N-terminal segment of t antigen to the same splice-acceptor sequence as is used in t mRNA. The result is the elimination of the t-antigen stop codon and the fusion, in a compatible reading frame, of two protein-coding segments;

translation of this RNA generates the mosaic T antigen. This is a simple example. The characterization of adenovirus RNAs by a large number of groups has revealed a dazzling spectrum of gene products generated by both differential use of promoters and differential splicing (see review by Ziff *Nature* **287**, 491; 1980).

Viruses pay a premium to keep their genomes small, and overlapping genes are an excellent way to compress a lot of information into a little space (as in the smaller bacteriophages such as Φ X174). There is little indication that higher organisms have gone to much trouble to trim down the size of their genomes. The average *Nature* reader has 3×10^9 base pairs of DNA per genome, give or take the odd supernumary chromosome, while salamanders, whose aspirations are significantly more modest, possess up to 10^{10} base pairs of DNA per haploid genome. Since it is believed that only about one-tenth of our DNA is used to encode proteins, one might predict that overlapping genes would be restricted to the viruses and that the host genomes would use the more familiar path of gene duplication to generate the same type of diversity. Not so, says α -amylase.

The story runs approximately as follows, with certain details omitted for simplicity.

The primary structures of murine liver and salivary gland α -amylase mRNAs have been compared by sequence and *S₁* nuclease/hybridization analyses, and this has been described in a recent issue of *Nature* (Hagenbüchle *et al. Nature* **289**, 643; 1981). These mRNAs are essentially identical except for the 5'-untranslated region, which is 206 nucleotides long in the case of the major liver species, but only 95 nucleotides in the salivary gland mRNA. Comparison of these sequences shows that both liver and salivary gland mRNAs share the 48 nucleotides of the 5'-untranslated region immediately adjacent to the coding region.

The secret of how this mosaic structure is

generated was revealed by a structural analysis of the chromosomal α -amylase genes. Young, Hagenbüchle and Schibler (*Cell* **23**, 451; 1981) cloned the α -amylase genes, located the mRNA-coding segments of their clones and determined the DNA sequence of the 5' areas of the gene; the region described in the current study covers approximately the 5' one-fifth of the mRNA-coding region. It turns out that the liver and salivary gland α -amylases are encoded by the same mosaic gene. Four exons have been found so far (there are presumably several other exons in the 3' regions of the gene, but these have not been analysed in mouse; for the rat, see MacDonald *et al. Nature* **287**, 117; 1980) which correspond, respectively from 3' to 5', to an internal coding region (called exon 4 here), the 5'-terminal coding region plus the 45 nucleotides of the 5'-untranslated region (exon 3), common to both mRNAs, and two alternative 5'-terminal exons — one of 161 nucleotides (exon 2), which is utilized in liver α -amylase mRNA, and one, about 2.8 kilobases further to the 5' side in chromosomal DNA, of 50 nucleotides (exon 1) which is used for the salivary gland mRNA. The two alternative mRNAs are probably obtained by differential transcription and/or processing but these points remain to be elucidated. There are promoter-like TATAA boxes at the usual 24 (± 1) nucleotides upstream from both exons 1 and 2 — it is plausible⁵ (see Young *et al. op. cit.*), therefore, that transcription initiates at exon 1 in the salivary gland and exon 2 in the liver. Equally, transcription could initiate at both sites and only the mRNA containing the appropriate leader might be exported to the cytoplasm in the relevant tissue. Whatever the mechanism, the uncapped liver-type leader sequence (exon 2) must presumably act as an intron in the salivary gland transcript; differential splicing must ultimately generate tissue-specific mRNAs with exons 1, 3 and 4 being spliced in the salivary gland and 2, 3 and 4 being spliced in the liver. As Young and his colleagues point out, this type of situation also exists in the immunoglobulin genes where multiple J regions exist in the active,

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rearranged, K-chain gene, for example; only one of these is destined to appear in the mRNA, the remainder being removed by differential splicing.

Why does the mouse go to all this trouble? Any explanation should consider the fact that α -amylase mRNA accounts for 2 per cent of the cytoplasmic mRNA in the salivary gland, but only 0.02 per cent in liver. This level may reflect the transcription rate from the two genes; the 'salivary gland' promoter would then be considerably stronger than the 'liver' promoter. Alternatively, the rate of RNA processing and/or export to the cytoplasm may be different for the two mRNAs. Another clue may reside in the fact that the liver mRNA has a much longer 5'-untranslated

region than the salivary gland mRNA. It is possible that this affects the translation efficiencies of the mRNA (see 'Discussion' in Young *et al.*). Although the explanation for this fascinating genetic mechanism is the subject of future work, it is, of course, likely to be tied up with the primary question — what determines the different level of α -amylase in two different tissues?

In the rat (and other mammals) the situation may be even more complicated. MacDonald and his co-workers (*Nature* 287, 17; 1980) have analysed the rat α -amylase genes and these studies point to at least five non-allelic α -amylase genes or pseudogenes. The further elucidation of the expression of the α -amylase gene family remains a mouth-watering prospect. □

Antibody-mediated enhancement of rabies virus

from J.S. Porterfield

THE long incubation period following naturally acquired rabies virus infection allows time for manipulation of the host's immune system in ways that can promote protection. Louis Pasteur's treatment of Joseph Meister was dramatically successful almost a century ago, and since then, very many others have benefited from rabies vaccines¹. There are, however, occasional failures, even when vaccine of established potency has been used, and much remains obscure about the pathogenesis of rabies virus infection, and about the precise way in which rabies vaccine confers protection. One puzzling effect, seen in both monkeys and mice immunized with rabies vaccine and subsequently challenged with rabies virus, has been termed the 'early death' phenomenon, since some vaccinated animals die sooner than non-vaccinated controls given the same challenge^{2,3}. Following earlier work which indicated that both bone marrow-derived B lymphocytes and thymus-derived T lymphocytes contributed to the clearance of rabies virus from the central nervous system^{4,5}, Prabhakar and Nathanson now present evidence, on p. 590 of this issue of *Nature*, that 'early death' in mice is mediated through antibodies, and is not a cell-mediated effect involving T lymphocytes; they do not, however, explain the mechanism whereby antibody produces this paradoxical response.

Apart from rabies, there are a number of other infections in which antibodies appear to produce effects which are detrimental to the host. The increased risk of severe bronchiolitis and pneumonia seen in infants who had been vaccinated with killed respiratory syncytial virus vaccine

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and subsequently acquired natural infection with that virus is well documented, but still inadequately explained⁶. Dengue viruses normally produce a non-fatal illness with a rash, but a proportion of children in South-East Asia develop haemorrhagic manifestations and may die from shock⁷. Although the effector mechanism responsible for the shock remains controversial, several laboratory studies related to haemorrhagic dengue are relevant to the 'early death' phenomenon. Monkeys inoculated with dengue virus mixed with sub-neutralizing concentrations of anti-dengue antibodies show higher levels of viraemia than control animals given dengue virus only⁸. Dengue virus replicates poorly in normal human or monkey peripheral blood white cell preparations, but shows enhanced yields of virus when exposed to infectious virus-antibody mixtures⁹. This antibody-mediated enhancement of viral replication is not restricted to dengue virus, but has also been demonstrated in continuous cell lines of mouse and human macrophages with West Nile virus¹⁰, with other Togaviridae and Bunyaviridae¹¹. Halstead first suggested that the mechanism involved in the human monocyte system depended on an increased uptake of virus complexed with antibody through Fc receptors (present on these cells but absent from most other cells), the internalized virus escaping destruction by the phagocytes and replicating within them⁹. Using the simpler model system with mouse macrophage-like cell lines, Peiris *et al.*¹¹ showed that the antibody-mediated enhancement of West Nile virus replication was specifically blocked by monoclonal antibodies directed against Fc receptors on the cells they were using.

Are these findings at all relevant to the rabies 'early death' effect? Rabies viruses were thought to be serologically identical, but a number of rabies-related viruses are now known¹², and antigenic variation between rabies virus strains has been established¹³. Mice inoculated with Lagos Bat or Mokola viruses, two of the rabies-related viruses, and subsequently challenged with rabies virus, also died more quickly¹⁴. These findings suggest that it is not essential to have homologous neutralizing antibodies to produce the rabies 'early death' effect, but that cross-reacting sera may also be active in this system as in the dengue system^{10,15}. Three monoclonal antibodies directed against the envelope glycoprotein of West Nile virus will all enhance West Nile virus although only one of these has neutralizing activity¹⁶. Monoclonal antibodies against known components of rabies virus and against rabies-related viruses are available, and it would be of great interest to determine which of these was capable of producing the 'early death' effect with rabies virus.

It might be objected that rabies virus replicates in the central nervous system in cells that would not have Fc receptors, so that the mechanism of opsonization of virus-antibody complexes could not apply. Rabies virus is capable of replicating in a wide variety of different cell types in addition to neurones, and it is certainly possible that macrophages within the brain or spinal cord might support replication, following 'enhanced' uptake in the presence of anti-rabies antibodies, although experiments with mouse peritoneal macrophages lend little support to this view¹⁷.

These comments in no way detract from the unquestionable efficacy of present post-exposure treatments of rabies, which combine the use of human diploid cell vaccine with human rabies immune globulin¹⁸⁻²⁰. They emphasize a continuing need for investigation of the complex interactions involved in the pathogenesis of rabies virus infection. □

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Antibodies, introns and biosynthetic versatility

from Miranda Robertson and Mike Hobart

SOON after the discovery that eukaryotic genes may be interrupted by non-coding DNA sequences, Gilbert¹ speculated in *Nature* that this unexpected arrangement might make it possible to produce variants of a single protein by differential splicing of the interrupted RNA. Specifically, he suggested that such a mechanism might underlie the well defined sequential changes that take place in the immunoglobulin molecule in the course of B lymphocyte ontogeny. As a result of these changes, a single antigen-binding site is associated successively with different effector sites to produce first immunoglobulin M, then immunoglobulin D, and later immunoglobulin G or sometimes A or E. Each of the different classes of immunoglobulin is expressed first as a membrane-bound receptor molecule and later, on the terminal differentiation of the lymphocyte, as a secreted protein which is identical to the receptor in all but a few residues at its carboxy terminal.

Both the class of the immunoglobulin molecule and whether it is membrane-bound or secreted depend on the structure of the constant region of its heavy chains (see Fig. 1). Thus each heavy-chain variable region (V_H region) is associated in turn with a constant region (C_H region) coding for the μ , δ and γ , α or ϵ chains corresponding to IgM, IgD, IgG, IgA and IgE; and each of these chains may then undergo modification at its 3' end. It is now known that the class switch from IgM to IgG, IgA or IgE is mediated by translocation of the V_H region DNA with deletion of the C_H regions 5' to the one that is expressed². Deletion, however, cannot explain the switch from IgM to IgD, because some B cells, including a few tumour cell lines, synthesize IgM and IgD at the same time; nor is it an attractive explanation for the switch from membrane to secreted proteins,

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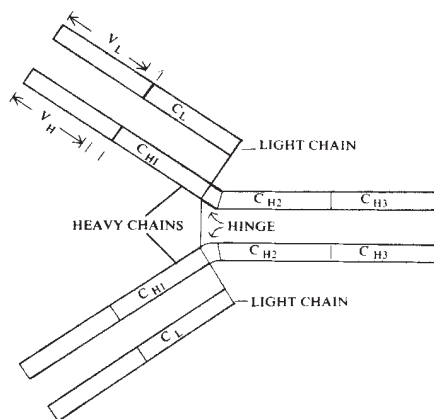


Fig. 1 Diagram of an antibody molecule showing the division of the heavy chains into the variable regions that determine antigen-binding specificity and the constant region domains. Each of the four domains, C_{H1} , C_{H2} , C_{H3} and the hinge, is encoded in a separate intron on the DNA. The sequences of the C_H introns are different for the different classes of immunoglobulin. The secreted form of each class (C_s) differs from the membrane form (C_m) in the last few carboxy terminal residues of C_{H3} : the membrane form has a longer and more hydrophobic carboxy-terminal which anchors it in the membrane; the diagram shows the secreted version.

which are also simultaneously expressed. Instead, as Gilbert surmised, these processes are controlled by processing of the RNA.

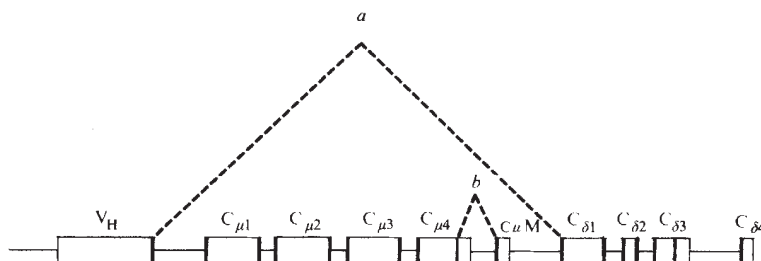
Immunoglobulin D biosynthesis has become accessible to investigation through the work of Liu and his colleagues³ who have located the gene coding for the corresponding δ constant region. The gene itself is very peculiar, having only two constant region domains (instead of the usual three) and an abnormal hinge region. Furthermore, it lies only about 2,000 base pairs from the μ gene corresponding to the IgM constant region; this is a much shorter distance than has so far been found to separate other constant region genes, and it means a single stable transcript could be

made from the combined μ and δ coding regions. There is, indeed, now preliminary evidence for the existence of such a transcript⁴. The switch from μ to δ could thus be mediated by the removal of the entire μ gene as an intron (see Fig. 2). The simultaneous expression of μ would be due to simultaneous production of shorter transcripts, ending before the δ sequences, or to polyadenylation at a site between the μ and δ genes.

This scheme has more recently been strongly reinforced by Moore *et al.*, who have confirmed the location of the δ gene in DNA both from mouse sperm and from a mouse hybridoma expressing membrane-bound forms of IgM and IgD, and established that the μ gene is not rearranged in the hybridoma DNA⁵. At the same time, however, they have raised the possibility that plasma cells committed to the secretion of IgD, unlike B cells transiently synthesizing the membrane form, may delete the μ sequences. So far, the evidence rests on a single rat plasmacytoma which secretes IgD and seems to have deleted the μ gene. Whether the deletion of μ is really a normal concomitant of the terminal differentiation of IgD-secreting lymphocytes, or whether it simply reflects the notorious instability of constant region genes in cultured cells, it is too early to say. It is not yet known, for example, whether the specialized switch sequences characteristic of the regions on the 5' side of the μ and γ genes² are to be found between the mouse μ and δ genes; but Rabbitts *et al.*⁶ have sequenced most of the region between the human μ and δ genes without discovering any.

The deletion or otherwise of the μ gene has, however, no direct bearing on the switch from membrane to secreted IgD, which is presumed to occur by the mechanism recently established for IgM. Independent observations by Alt *et al.*⁷ and Rogers *et al.*⁸ show that cells which are making both membrane and secreted IgM

Fig. 2 Diagram of chromosomal DNA coding for IgM and IgD in a B cell. The open rectangles are exons and the intervening solid lines represent introns. Heavy vertical bars represent RNA splicing signals. In cells expressing both membrane IgM and membrane IgD, it is believed that the entire region is transcribed. Dotted line *a* joins the introns that are spliced to produce membrane IgD, with the elimination of all the C_μ introns. Dotted line *b* joins the introns that are spliced to produce the membrane form of IgM. Note that if the switch from membrane to secreted IgM occurs through termination of transcription after the μ_s segment, transcription of the δ gene will also be shut off. The switch from membrane to secreted δ chains is believed to occur as described for μ .



contain μ chain messenger RNAs of different lengths. By examining cells in which the two forms of μ chain are synthesized in different proportions, Alt *et al.* were able to deduce that the longer of the two messages codes for the membrane (μ_m) form. Rogers *et al.* hybridized the two messages with cloned genomic DNA containing the μ gene, and found that while both hybridize the coding sequence of the four major μ domains, the longer one also hybridizes with a short segment on the 3' side. The same team went on to show⁹ that this short segment codes for a hydrophobic protein sequence whose characteristics are similar to those of known membrane-bound proteins.

Thus the secreted form of the protein (μ_s) is encoded by the four main μ gene segments, while the expression of the membrane form entails the addition of a further segment at the 3' side. This 3' μ_m segment has a sequence characteristic of a splice site at the beginning of an exon at its 5' end; but the matching splice sequence upstream occurs within the coding sequence of the C μ 4 domain, followed by the coding sequence for the last 19 amino acids of the μ_s chain and a termination codon. Early *et al.* suggest on the basis of this organization that μ_m , the first antibody to be synthesized by a B cell, is produced from a long transcript extending to a polyadenylation site beyond the μ_m coding segment. In the course of the processing of the transcript to mRNA the μ_s coding segment is eliminated as an intron, with the consequence that the termination codon is also removed.

When the B cell matures into an antibody-secreting plasma cell, either transcription terminates upstream of the μ_m segment or, for some reason, the μ_m segment is cleaved from the transcript. In either case the μ_s sequence is retained in the mRNA and read until the termination codon.

Thus the class switch from IgM to IgD seems to work by a mechanism analogous to that which operates the switch from μ_m to μ_s ; and it has, incidentally, been pointed out that the μ_m - μ_s switch may automatically eliminate the production of IgD by IgM-secreting cells if transcription ends at the μ_s segment (see Fig.2).

The organization and regulation of the δ coding sequences open two further questions, the first specifically about the δ chain and the second generally about RNA splicing.

The first arises from the fact that the

coding sequences as described by Liu *et al.* can account for the shorter of the two known versions of the δ chain but not for the longer version¹⁰. However, further sequences apparently coding for the δ chain have more recently been detected (but not yet characterized)¹¹ further downstream, lending weight to the proposal of Liu *et al.* that differential splicing may provide a family of different δ chains with different — and it must be confessed entirely unknown — functions.

The second question is that of the mechanism by which introns are eliminated

from nuclear RNA. Until now, the simplest scheme — that the splice site at the end of each exon is paired with the matching sequence at the beginning of the next one downstream — has also been consistent with the known data. However, it plainly cannot account for the removal of the μ exons from the large μ - δ transcript. There is still a formal possibility that such a combined transcript does not exist; but if it does, it will clearly represent a further strong incentive to molecular geneticists of immunoglobulin to devise a splicing system that will work *in vitro*. □

Anomalous sputtering at high energy

from P.K. Haff and L.E. Seiberling

WHENEVER a sufficiently energetic stream of particles impinges on a target, there is some probability that target atoms and molecules will be knocked loose from the surface. This process of erosion on an atomic scale is called sputtering. For incident ions in the keV per AMU range, the average number of target particles ejected per ion is called the sputtering yield, S . Values of S in the range 1 to 10 are common. Quantitative calculations of the sputtering yield in this regime are based on the idea of a collision cascade (a primary recoil target atom makes collisions which produce secondaries which in turn produce tertiaries, and so on), and predicted yields typically agree with experiment to within a factor of 2 or 3.

At higher-incident ion energies, in the hundreds of keV per AMU to several MeV per AMU range, for example, the collision cascade predictions for the sputtering yield are small compared with unity. However, recent experiments using high-energy ion beams have shown that for some materials very large sputtering yields ($S \sim 4,000$ in some cases) exist when the collision cascade prediction is $S \ll 1$.

At Bell Laboratories, Walter Brown and his collaborators^{1,2} have shown that H and He in the 100 keV per AMU range can sputter H₂O ice at rates up to three orders of magnitude faster than those predicted according to the low-energy collision cascade calculations. Moreover, at temperatures below ~ 100 K, the yield is independent of temperature, and appears to be proportional to the square of the electronic stopping power, $(dE/dx)_e^2$. This is to be contrasted with the energy dependence of the collision cascade, which is given by the nuclear stopping power $(dE/dx)_n$ describing the energy loss of the projectile to elastic atomic collisions. Evidently energy is transferred from the initial electronic excitation into kinetic

energy of atoms, some of which escape as sputtered particles. Brown and his colleagues favour an explanation based on the ion explosion model. Neighbouring atoms, partially stripped of their electrons by the passage of the projectile, acquire kinetic energy through their mutual Coulomb repulsion. Such a picture leads essentially to a $(dE/dx)_e^2$ prediction for the energy dependence.

An early prediction³, that in certain dielectrics the ion explosion mechanism would lead to an anomalously high sputtering yield essentially proportional to $(dE/dx)_e^2$, led to a series of experiments in the Kellogg Radiation Laboratory at Caltech by Joseph Griffith⁴ and Elizabeth Seiberling⁵. These workers utilized beams of heavy ions (¹⁶O, ¹⁹F, ²⁰Ne) to bombard a dielectric target, UF₄. Sputtered ²³⁵U atoms were collected on mica, fissioned with thermal neutrons, and the resulting fission damage tracks in the mica counted to determine the yield S . This sensitive technique⁶ has allowed the Caltech group to determine the energy spectrum of sputtered particles as well as the dependence on bombarding energy. They discovered that, for ¹⁹F on UF₄, the excitation curve had a much steeper dependence on energy than $(dE/dx)_e^2$, and that the energy spectrum of sputtered particles was essentially thermal at a temperature of 3,500K. To explain their data Seiberling and co-workers have proposed a 'thermalized' ion explosion model, wherein Coulomb energy of neighbouring ion pairs is transformed into random thermal kinetic energy of atomic motion of particles located along the ion path. The model correctly gives the strong energy dependence of the yield, as well as the energy spectrum.

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The discrepancy between the energy dependence of S , as measured by the two groups, does not necessarily mean that one is right and the other wrong. The assumptions underlying the thermalized ion explosion picture may breakdown for H and He bombardment where the ionization per unit length along the projectile track is considerably smaller than in the UF_4 experiments involving heavy ion projectiles.

Regardless of the precise nature of the mechanisms involved, enough has been learned to know that anomalous sputtering is probably quite important in the Solar System. Extrapolating Pioneer spacecraft data and the Bell Laboratories H_2O sputtering results, Lanzerotti and colleagues⁷ pointed out that over geological time significant erosion can be expected to have occurred on the icy satellites of Jupiter. They argued that up to 1 km of ice may have been lost to space, that interplanetary ice grains would be destroyed in tens of thousands of years by sputtering by the solar wind⁸, and that the ice particles comprising the rings of Saturn may be substantially affected by magnetospheric proton bombardment⁹. Work at Yale and Caltech has focused on the rate of erosion by sputtering of surface features, such as meteorite craters¹⁰, on the sputter-induced formation of planetary atmospheres¹¹, and on the albedo changes of icy satellite surfaces due to differential sputtering of ice and embedded silicate grains^{12,13} (which do not exhibit anomalous sputtering in the energy range considered).

It is clear that there are a large number of potential applications to problems in planetary science. The range of experiments reported so far, however, is relatively small, so, with theoretical ideas requiring further development, much uncertainty is attached to calculations of planetary processes. The Caltech energy spectrum suggests that energies of sputtered particles peak in the 0.3 eV range. This is substantially less than the energy required by, for example, an O atom to escape into space from Ganymede. Will H_2O exhibit a similarly soft spectrum? Probably it will, but until one can measure, or know enough of the mechanisms to estimate reliably, such quantities, it will be difficult to assess with confidence the detailed response of satellite surfaces to ion bombardment. □

Spinning asteroids

from David W. Hughes

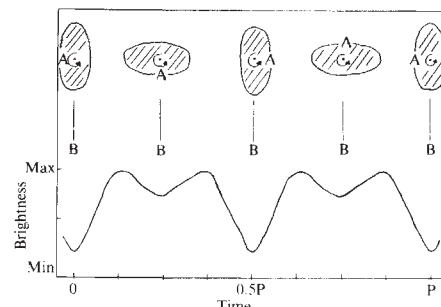
ASTEROIDS spin with periods which usually lie between 5 and 12 hours although they can vary from 4 to 50 hours. The values are obtained from observations of fluctuations in brightness. Large variations are caused by gross changes in the projected cross-sectional area as seen from Earth (see the figure) and smaller variations by changes in the surface properties. Over longer time periods, of the order of a year or more, the light curve shape alters according to variations in the aspect angle — the angle between the line of sight and the asteroid's spin axis. Ideally these long-term variations should lead to an estimation of the asteroid's shape.

Problems arise when interpreting the results because too few asteroids have been observed and observational selection effects must be removed. These problems have been tackled by E.F. Tedesco, (Lunar and Planetary Laboratory, University of Arizona) and V. Zappalà (Osservatorio Astronomico di Torino, Italy) in their recent paper in *Icarus* (43, 33; 1980).

About 2,150 asteroids are known and of these 321 have known spin periods. Tedesco and Zappalà divide these into two groups — 187 are members of families and the rest are main belt non-family asteroids. Previous studies in 1973 and 1979 were based on a total sample of only 64 and 182 asteroids respectively, so from this aspect alone Tedesco and Zappalà's work is a considerable improvement.

There are several fundamental selection effects in asteroid light curve data. Two main asteroid classes exist — C types which have a low albedo and are carbonaceous and S types which have a higher albedo and are siliceous or stony-iron in composition. Selection favours asteroids of high apparent brightness, which is equivalent to favouring the largest and nearest and the ones with highest albedo. Unfortunately the C to S ratio varies both as a function of size and distance from the Sun. Apollo and Amor asteroids are also unusual. These have orbits which cross those of Earth and Mars. Their mean rotational brightness amplitude is about 0.5 magnitudes while comparable-sized main belt asteroids have amplitudes usually less than 0.2 magnitudes. Members of the Appolo/Amor group are clearly more irregular and probably have a different evolutionary history to main belt asteroids. Another selection effect is inherent in the photographic photometry used to measure small asteroids. Usually observing periods of only 6 hours can be employed and variations of less than 0.2 magnitudes cannot be detected. Sixty per cent of the observed asteroids do not reveal their spins, indicating that the spin period is too long or the asteroid too regular. A further complication results from the fact that

many small asteroids were specifically selected for observation simply because they were members of Hirayama families. The family members have similar orbits and probably resulted from the fragmentation of a single parent. Removing the selection effects reduces the original data set of 321 to a final set of 134.



A theoretical light curve for a spinning asteroid showing how the brightness varies as a function of time. P is the period. The spin axis is perpendicular to the paper and the asteroid is viewed from B. Position A represents a fixed place on the asteroid.

Tedesco and Zappalà find that there is no clear-cut trend towards faster rotation for smaller objects. This is contrary to the conclusions of previous researchers who regarded collisional fragmentation as an important contributor to the modification of spin rates. While no definite relationship exists, the authors stress that the distribution is not entirely random. Asteroids larger than 175 km have a mean rotation period near 7 hour compared with a 10-hour period for smaller ones.

Considering asteroids with sizes between 75 and 175 km they found that the S asteroids have a mean period of 9.4 hours whereas the C asteroids have a period of 11.6 hours. The difference between these values is only 1.6 times the standard deviation and the authors conclude that this is too small and thus the spin rate is not a function of type.

How do asteroid shapes vary as a function of size and compositional type? It has been shown that the spin axes of asteroids are fixed in space and that it only takes about one million years to align the spin axis with the axis of maximum moment of inertia. Studies also indicate that asteroid surfaces generally lack variegation in colour or albedo. Unfortunately, shapes cannot be deduced directly from light curve amplitudes. Tedesco and Zappalà conclude that the very largest asteroids (diameter $D > 300$ km) have very low-amplitude variations (0.11 ± 0.02 magnitude). There seems to be

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an abrupt increase in amplitude as the diameter decreases below 200 km (to about 0.21 ± 0.01). Even so, the shapes of smaller asteroids do not become significantly more elongated with decreasing size.

A search for possible collision-induced effects revealed that asteroids found in the zones of the asteroid belt which have low number densities do seem to have a higher value for the amplitude variation. Unfortunately the relative numbers of the specific asteroid types vary as one moves from zone to zone and this further complicates the picture.

When it comes to asteroid families it seems that the smaller members of the families have higher spin rates than normal. Larger family members ($75 < D < 175$ km) are virtually indistinguishable from non-family asteroids.

The significant conclusion of this investigation is that there is a pronounced

change in asteroid rotational properties at about 200 km. Asteroids larger than this seem to be primordial (at least in the sense that they are not remnants of a larger disrupted parent body), nearly spherical and probably have prograde rotation. Smaller asteroids have random spin axis orientations and the population contains a higher proportion of slowly rotating objects. It is interesting to note that the dividing diameter of 200 km is strongly evident when the frequency-size relationship of asteroids is investigated. The mass distribution index of asteroids changes at this diameter.

Future progress in the study of asteroid spin statistics and the effect of collisional evolution depends on the acquisition of more data. The picture will be a lot clearer if the number of asteroids with accurately measured spin periods could be increased by a factor of two or three. □

and PRL mRNA in cultured pituitary cells after treatment with EC. The very good correlation between the two suggests that EC acts either at transcription, RNA processing or RNA breakdown. It is slightly odd that the decreases occur over 20–100 hours which suggests that the mRNA may have a fairly long half life. Interestingly though, EC affects PRL specifically (no other pituitary hormones are affected) and is 100 times more effective than is dopamine.

This picture is, however, somewhat confused by the finding that BEC increases the rate of breakdown of the hormone itself (Dannies and Rudnick *J. biol. Chem.* **255**, 2776; 1980). Maurer (*Biochemistry* **19**, 3573; 1980) suggests that BEC blocks PRL secretion by the cells and the subsequent accumulation inside the cells results in increased proteolysis by lysosomal enzymes. The fact that chloroquine (which reduces the rate of appearance of lysosomal enzymes in the cytoplasm) partially reverses this effect of BEC favours such an interpretation.

Hormone production can also be prevented by incubating the pituitary cells with hydroxynorvaline — an analogue of threonine which can be incorporated into protein. Normal processing of preprolactin to PRL involves cleavage at the bond between residues 30 (normally threonine) and 31. Apparently the processing enzyme cannot hydrolyse this bond when threonine is substituted by hydroxynorvaline because the prehormone accumulates in the analogue-treated cells (Hortin and Boime *J. biol. Chem.* **255**, 7051; 1980).

Rossier and colleagues (*Proc. natn. Acad. Sci. U.S.A.* **77**, 666; 1980) have shown that stress induces PRL synthesis and they believe that this arises from the stress-induced production of opiate peptides which are known to increase PRL levels.

More recent results (Denef *et al.* *Nature* **285**, 243; 1980) have suggested that the action of dopamine is not quite as simple as was previously thought. Although high levels ($\geq 1 \mu\text{M}$) of dopamine do indeed reduce PRL, at much lower levels ($\leq 1 \text{ nM}$) dopamine actually stimulates PRL production.

The nature of the interactions between prolactin and the steroid hormones is still not clear. Although oestradiol seems to stimulate PRL production, its metabolite, 2-hydroxyoestrone, produces a rapid decrease in PRL levels (Fishman and Tulchinsky *Science* **210**, 73; 1980). The hydroxylating enzyme is inhibited by the opiate agonists which stimulate PRL release and is activated by antagonists which suppress PRL. This means that *in vivo* the opiates may affect PRL levels by their effect on the enzymes which metabolize the steroid hormones.

Clearly we still have some way to go in our attempts to understand how the levels of this interesting molecule really are regulated. □

Production and processing of prolactin

from Alan D.B. Malcolm

THE pituitary hormone prolactin (PRL) was for many years something of an enigma. Its similarity to growth hormone both chemically (human PRL and human GH have 40 per cent of their amino acids in common) and biologically (in lower mammals PRL acts as a growth hormone) meant that its very existence in humans was disputed. Advances in protein purification and radioimmunoassay have now resolved this question and we are beginning to understand something about the factors controlling its synthesis and breakdown. Controls seem to exist at many stages including transcription, RNA processing, translation, preprolactin processing and secretion together with nuclease degradation of RNA and proteolytic attack on the various polypeptides.

The mRNA for rat PRL was obtained by extracting the poly(A)-containing RNA from the pituitary after stimulation of the rat with diethylstilboestrol (Gubbins *et al.* *Nucleic Acids Res.* **6**, 915; 1979). This RNA was used to produce cloned copy DNA, which has since been used to identify genomic PRL sequences in a rat genome 'library'. The PRL gene contains four intervening sequences (Chien and Thompson *Proc. natn. Acad. Sci. U.S.A.* **77**, 4583; 1980), one of which occurs in the 5'-untranslated region. The sequence of one of the genomic clones (Gubbins *et al.* *J. biol. Chem.* **255**, 8655; 1980) shows that some of the intron sequences are repeated elsewhere in the rat genome. It is interesting that although the coding lengths of PRL and GH are similar and they both have four introns, the lengths of the introns are much

greater in PRL than in GH. The total length of the PRL 'gene' is therefore 10 kilobases compared with only 2.1 for GH.

Maurer and his colleagues (*J. biol. Chem.* **255**, 2243; 1980) have approached the problem of transcription and processing of PRL RNA by isolating RNA from the nucleus and cytoplasm of pituitary cells. The RNA was then electrophoresed through an agarose gel and transferred to diazobenzylxymethyl paper. Hybridization of the now immobilized RNA to ^{32}P -labelled cDNA revealed the presence of five PRL bands (7.0, 6.4, 3.8, 1.7 and 1.0 kilobases) in nuclear RNA but only one (1.0 kilobase) in cytoplasmic RNA. Presumably the different nuclear RNA species arise from ordered removal of the introns.

The secretion of all the pituitary hormones is controlled by the hypothalamus. In the case of PRL, dopamine is probably an important inhibitory factor and many dopamine agonists, such as the ergot alkaloids ergocryptine (EC) and its synthetic congener bromoergocryptine (BEC), will reduce overall PRL production. EC is used clinically to reduce PRL levels in spite of slightly unpredictable side effects (*New Engl. J. Med.* **302**, 749; 1980).

To investigate the stage at which EC regulates PRL production, Maurer (*J. biol. Chem.* **255**, 8092; 1980) has studied the decrease in both *de novo* PRL synthesis

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Tropical soils and agrotechnology transfer

from Ian Smalley

MORE food needs to be produced in the third world, in the countries which are grouped in the tropical regions. But the soils of the tropics often have a 'variable charge' character which makes them sufficiently different from most soils of the temperate regions that research in the developed world can not be directly applied. The transfer of agriculture technology between the developed and the developing world can thus encounter fundamental problems.

Various aspects of variable charge soils, and in particular the problems of classification and of transfer of agricultural technology, were discussed at a recent meeting* at Massey University in New Zealand. In variable charge soils the electrical charge on the particle surfaces, both in magnitude and sign, can vary greatly with changes in the pH, ionic strength and composition of the soil solutions. The variable charge nature of the soils arises from the abundance, in the clay fraction, of minerals having amphoteric (positive or negative) surfaces. Prominent among such minerals are the oxides and hydrous oxides of aluminium and iron which may be crystalline, paracrystalline or poorly ordered. The 1:1 type layer silicates, such as halloysite and kaolinite, also show amphoteric properties, associated with their relatively large exposed crystal edge surface. On the other hand, the negative surface charge of 2:1 clay minerals arises from extensive isomorphous substitution within the structure and hence is largely independent of pH. Thus soils rich in micaceous and similar minerals are said to be of constant or permanent charge.

Besides having amphoteric surfaces, clay-size mineral constituents with variable charge generally have a low capacity to adsorb or retain nutrient cations in the range of pH and ionic conditions which prevail in natural soil systems. For this reason, these constituents have been grouped together and referred to as 'low activity clays'. Thus, soils rich in low activity clays are, in general, naturally infertile and this causes a major problem in tropical agriculture. Although the amphoteric nature of some soils and soil constituents has been recognised for a long time, it is only relatively recently that its importance, in terms of soil processes and management, has come to be appreciated. This is because soil research, as carried out in the developed countries of Europe and North America, has largely been directed to understanding the behaviour of temperate soils, many of which are of the

constant charge type. The variable charge constituents, if they were not altogether ignored, were often regarded as contaminants and removed from the system.

A common constituent of volcanic variable-charge soils is the clay mineral allophane (now widely believed to have a spherical structure: see *News & Views*, *Nature*, **281**, 339, 1979). Recent studies on allophane in New Zealand soils were reported by R. Parfitt (NZ Soil Bureau). Allophane has been defined as a series of naturally occurring hydrous aluminosilicate clays, characterised by short range order and by the predominance of Si-O-Al bonds, and it appears to be made up of hollow spherules 3.5–5.0 nm in diameter. Spherules with a molar ratio close to Al/Si = 2.0 from Mt Egmont andesitic pumice have the proto-imogolite infrared spectrum. This, together with X-ray fluorescence, pyridine adsorption and silicate analysis data, indicate that the wall of the allophane spherule is made up of imogolite segments. The fine tubular crystals of imogolite are also found in New Zealand soils.

Allophane (Al/Si = 2.0) occurs in several different environments in New Zealand. It is the dominant clay mineral in an Egmont soil formed from andesitic ash, in a Taupo soil formed from rhyolitic Taupo pumice, and in a Mairoa soil formed from rhyolitic ash. In rhyolitic ash, allophane (Al/Si = 2.0) is the dominant clay mineral where the annual rainfall exceeds about 1,600 mm. Where the annual rainfall is less than 1,600 mm, allophane and halloysite are the dominant clay minerals, with allophane decreasing and halloysite increasing in older tephra. Halloysite is the dominant clay mineral in a Kereone soil, where the rainfall is 1,200 mm.

Allophane soils present a problem for mechanical analysis and clay recovery because at field pH or in acid media the positively charged allophane flocculates with any crystalline clay present by charge interaction, and in alkali or calgon solution it is self-flocculating by some unknown mechanism. Studies of Scottish allophanic subsoils by H.J. Fullerton (University of Glasgow) showed that these behave like New Zealand allophane soils in that they flocculate by charge interaction when vibrated in water and continue to flocculate at all alkali pH values. Unlike allophane soils, however, they deflocculate in calgon solution. Alkaline flocculation appears to be not a rare property confined to allophane, but a common property of disordered clay surfaces.

The major technology transfer project discussed, the Benchmark Soils Project, is being applied to three soil types across the tropical world. Three different soil families have been chosen for experimentation: the

clayey, kaolinitic, isohyperthermic Tropeptic Eutrustox (the terminology of the USDA-developed 'soil taxonomy' system is used); the thixotropic, isothermic Hydric Dystrandepts; and clayey, kaolinitic, isohyperthermic Typic Paleudults. These soil families represent three different agroclimatic zones: dry and warm (the Eutrustox), moist and cool (the Dystrandepts), and moist and warm (the Paleudults). The soils all have distinct variable charges which influence soil management and utilization. G. Uehara (University of Hawaii) reported that technology transfer in 15 separate areas was being attempted; among these were selection of tree crops for windbreak, fuelwood and erosion control, susceptibility of salinization, and soil workability and trafficability.

The Benchmark Soils Project comprises two companion research contracts awarded to the Universities of Hawaii and Puerto Rico in 1974 and 1975 by the US Agency for International Development. The primary objective of the project is to test the hypothesis that agro-production technology, particularly soil management experience, can be transferred among tropical countries on the basis of soil families as defined in the system of soil taxonomy. The project has established a research network that includes 23 experimental sites in Brazil, Cameroon, Hawaii, Indonesia, the Philippines and Puerto Rico. The basic research strategy is to select soils belonging to the same soil families in widely separated geographical areas, subject these soils to identical and standardized experiments, and correlate the plant yields. The transfer experiments are complemented by pragmatic management experiments and variety trials intended to expand the basic knowledge of crop production for each soil family.

R.L. Fox (University of Hawaii) pointed out that in their attempts to replace the humus theory of plant nutrition with the inorganic nutrient theory, early naturalists assumed that it was only necessary to replace the nutrients removed in the harvest. Phosphate reversion, K fixation, denitrification, soil erosion and leaching of nutrients were not sufficiently recognized. We now know that a soil fertility programme that is content with returning nutrients exported in the harvest is not sufficient at all. This fundamental mistake has been repeated almost endlessly during the past century — most recently in the tropics. Nowhere are the consequences likely to be so acute as on the weathered, variable charge soils which are abundant there. □

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*'Soils with Variable Charge' Conference: Commissions 4-7 of the International Society of Soil Science; 11-18 February, 1981. Conference Book (ed. B.K.G. Theng) publ. NZ Soc. Soil Sci. 1981.

Interfering plants

from Peter D. Moore

IN his classification of competitive strategies, Miller (*Adv. ecol. Res.* 4, 1; 1947; and *Brookhaven Symp. Biol.* 22, 63; 1969) recognizes two major categories, exploiters and interferers. The interferer has a large body size, a long generation time and is relatively independent of physical environmental factors. Rather than evolving efficient mechanisms for resource exploitation, they substitute space for the resource and succeed by means of their capacity for the physical exclusion of potential competitors from the resource. The concept was developed largely with certain vertebrate animals in mind, especially those exhibiting territorial behaviour.

There are also some members of the plant kingdom which deserve the title 'interferers'. In particular, those which assume dominance in stable ecosystems have characteristics which correspond to those of the interferer. The substitution of space for a resource may be achieved purely by physical growth, or may be achieved by more subtle means. The production of chemical inhibitors which curtail the growth of plant individuals of either the same or another species is one such mechanism. Even more subtle is the process described by Rice and Pancholy (*Am. J. Bot.* 59, 1033; 1972; see also *Nature* 254, 184; 1975) in which toxins from litter of grassland climax dominants is considered to inhibit the process of nitrification (the conversion of ammonium ions to nitrate in the soil) by suppressing the activity of the soil microbes *Nitrobacter* and *Nitrosomonas*. Such a strategy would reduce the rate of nitrogen recycling and thereby slow down further ecosystem growth and vegetation succession.

This attractive and sophisticated

interference technique has, however, received little supporting evidence over the past few years. It is difficult to distinguish between direct chemical suppression and rather more complex interactions involving the depletion of other necessary resources (for example, phosphorus) or the overall reduction of pH, both of which could influence the rate of nitrification. Jordan, Todd and Escalante have tried a technique of manipulative experimentation in the Amazonian rain forest (*Oecologia* 39, 123; 1979). They added calcium carbonate to the thick root mat on the forest floor and checked the leachate for nitrogen losses. They observed an enhanced loss of nitrogen, but it was mainly in the form of ammonium ions, so the treatment had not stimulated nitrification. They also noted that the pH of the drainage water was still as low as 4.5 and thus concluded that it was the low pH of the soil which was inhibiting the activity of the nitrifying bacteria. However, they conceded that, since the leachate had the colour and consistency of tea, it could be the tannin from the leachate which was responsible for the suppression.

The deciduous forests of Missouri have provided opportunities for further analysis of the curtailment of nitrification. Here Lodhi (*Am. J. Bot.* 64, 260; 1977; 65, 340, 1135; 1978) has shown a heterogeneous pattern in the nitrogen levels of soils under different canopy tree species. Ammonium ion concentrations were invariably higher than nitrate levels, the degree of excess being related to canopy species. Also, there was an inverse relationship between am-

monium ion concentration and the density of nitrifying bacteria in the soils. Under some trees, such as American elm (*Ulmus americana*) and sycamore (*Platanus occidentalis*), the soils were of neutral pH, so one cannot resort to a low pH explanation for the suppression of nitrification at all these sites.

Lodhi has now backed up his contention that polyphenolic compounds can be involved in these woodland inhibition observations by his studies with Killingbeck (*Am. J. Bot.* 67, 1423; 1980) on the ponderosa pine stands of North Dakota. Here the soil pH is neutral to slightly alkaline (mean pH 7.25 in the A horizon and 7.75 in the C horizon). Once again, the ammonium ions considerably exceed nitrate ions and the population of *Nitrosomonas* and *Nitrobacter* were low. He then added various extracts of ponderosa pine needles and bark to experimental cultures of these microbes and found that maximum inhibition of their activity (93 per cent) was achieved with aqueous extracts of the needles or with mildly alkaline soil hydrolysates. Both of the extracts were rich in condensed tannins, which probably accounted for the inhibition.

Nothing is known of the mechanism of nitrogen uptake by ponderosa pine but Lodhi speculates that there would be considerable ecological advantages in its taking up ammonium rather than nitrate ions. In this way leaching losses of nitrogen would be reduced, energy would be conserved and the tree would benefit in terms of competition for the soil nitrogen resources. More data is required before such speculations can be confirmed but, should they prove correct, *Pinus ponderosa* will effectively fit the Miller criteria defining an interferer. Whilst satisfying its own requirements, it denies other organisms access to the soil nitrogen resource by allelochemic inhibition of nitrification.

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100 years ago

THE NEW MUSEUM OF NATURAL HISTORY

The great terra-cotta building facing Cromwell Road, South Kensington, and occupying the site of the old 1862 Exhibition, about which for the past twelve months public curiosity has been raised, is about to draw up its blinds, and offer a part of its extensive galleries for inspection on Easter Monday.

It is no secret that for thirty years past the accommodation in the British Museum, Great Russell Street, Bloomsbury, "both for man and beast," had become too restricted, and the necessity for a larger building was keenly felt. As usually happens in such cases, the most adventurous and energetic officer was the first to obtain for his department what he

required, namely, more room.

Sir A. Panizzi (then Keeper of Printed Books) projected, shortly after the 1851 Exhibition, his scheme for a great central Reading-room and Library, and some years later on, the Department of Antiquities, represented by Mr. C. T. Newton, C.B., also obtained an addition to its galleries on the western side, and still more recently on the southern side, next to the great Entrance Hall.

Great praise is due to Mr. Bond, the present Principal Librarian, for putting an end to the use made of the fine colonnade in front of the British Museum, which for twenty-five years was blocked by antiquities covered in with a row of extremely unsightly and incongruous wood and glass sheds. These are now happily removed. The Department of Prints and Drawings lacking a gallery, obtained possession of the "King's Library" floor on the east side for an exhibition space; and even the conservative Coin Department likewise laid out for the public a few show-cases here of coins and medals.

But, like the clothes of the rising son, in the old caricature, the collections everywhere had outgrown their receptacle, and none more so than the departments of Natural History. Scientific men were however not unanimous, and a fierce controversy was carried on in 1858-59 as to the relative merits of enlargement on the old site, or dismemberment. Finally, after a Committee of the House of Commons

Erratum

In *A tropical Volute shell and the Icarus syndrome* from Jonathan C. Howard (*News and Views* April 9, 290, 441, 1981) 'simulate' was incorrectly printed as 'stimulate' on p442, column 3, line 37. The correct sentence reads "C.H. Waddington⁸ pointed out as long ago as 1942 that genetic modifications to adaptable systems can simulate the inheritance of acquired characters."

had taken evidence upon the subject, the removal of the Natural History Collections was decided upon by the Government.

But the death of the Prince Consort, the delay of the House of Commons to vote the necessary funds, the retirement of Sir A. Panizzi from the post of Principal Librarian, the discussion of rival plans, the inevitable delays about the completion of any Government building, caused twenty years to pass before the plans of the chosen architect, Mr. Alfred Waterhouse, were realised in a solid and material form.

From *Nature* 23, 14 April, 549, 1881.

REVIEW ARTICLE

Dynamics, diffusion and geomorphological significance of tidal residual eddies

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Many shallow tidal areas show the occurrence of ebb and flood surpluses, organized in cell-like structures or 'tidal residual eddies'. They seem to arise from a nonlinear transfer of vorticity from the oscillating tidal field to the mean field, the irregular structure of the bottom topography or the coastline acting as a catalyst to produce the necessary vorticity gradients. The dynamical characteristics of the eddies assist our understanding of the geomorphology of the sea bed.

VAN VEEN'S review¹⁻³ of the hydrography of the Straits of Dover and its influence on the morphology of the Dutch coast stimulated much research on the connection between tidal currents and a variety of coastal geomorphological features. Much attention has since been given to the occurrence of linear or parabolic sand ridges in tidal areas, such as the Flemish Banks in the Southern Bight of the North Sea. We now know that nearly all shallow tidal areas where oscillating currents exceed some critical speed (0.5 m s^{-1}) and where sand is present, are sites at which these ridges can be found (see Fig. 1)⁴. Tidal currents in the vicinity of these ridges are generally asymmetrical in that at some places more water seems to be displaced in the direction of the flood current (a flood surplus) whereas the reverse occurs elsewhere (an ebb surplus). These surpluses, called residual currents, are often organized in cell-like structures or 'residual eddies' (see Fig. 2). Yet, although these features have long been recognized, the underlying physical dynamics of residual eddies in connection with tidal sand ridges have only recently become understood.

The dynamics of residual eddies occurring in geomorphological conditions other than tidal sand ridges, such as the generation of residual eddies by headland promontories or by

narrow tidal inlets along the coast and the organization of ebb and flood surpluses by the gross geometry of half-enclosed tidal embayments, have also been extensively investigated. Although, at first sight, it seems that different mechanisms are at work in each geomorphological setting, it is shown here that the formation of residual tidal circulation can in each case be understood as a transfer of vorticity from the tidal oscillating current velocity field to the mean (residual) field, though the way in which tidal vorticity and tidal vorticity gradients are produced in the first instance differ.

Although we now have a good understanding of the fluid dynamical response of an irregular sea bed or coastline to the tidal current regime, we are still far from having a satisfactory theory for the interaction of tidal currents and morphological structures concerned, that is, a complete explanation of how the morphology is both being shaped by, and gives shape to the current velocity field. However, the recently developed physical description of tidal residual circulation gained by observations in the field, by hydraulic or numerical modelling and, principally, by theoretical analysis may provide a basis for such a theory and may also help our understanding of the contribution of residual eddies to diffusive transport processes in tidal areas.

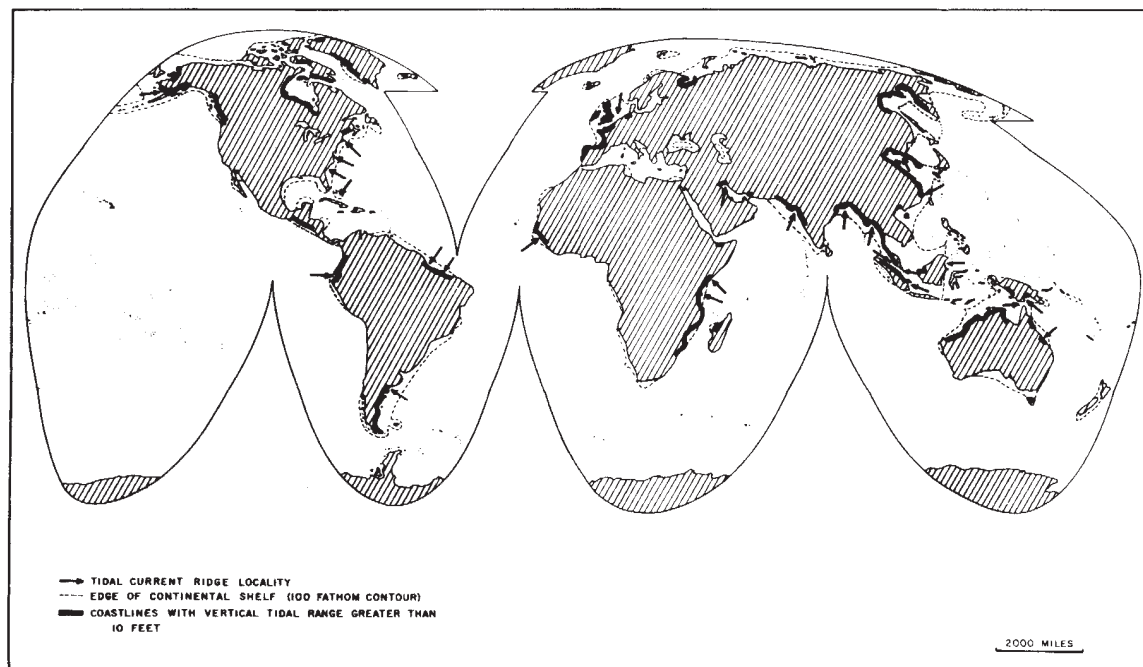


Fig. 1 The worldwide distribution of tidal current ridges according to ref. 4.

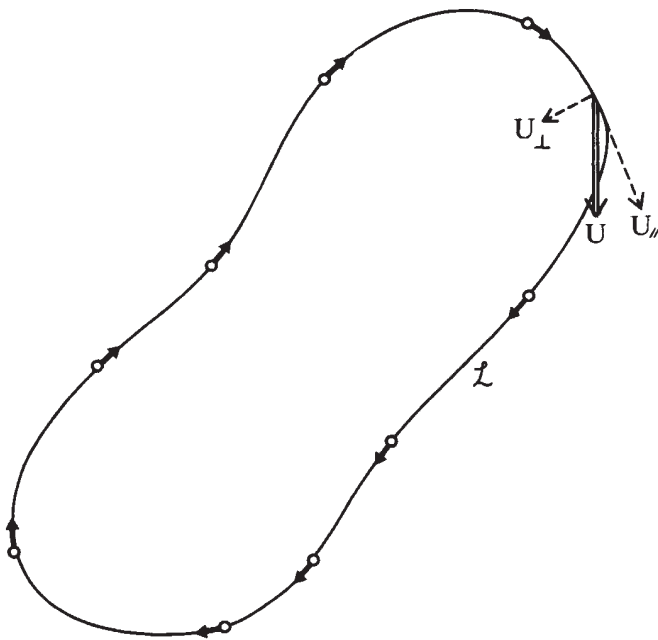


Fig. 2 A string of current meters produces, after averaging the instantaneous velocity $\mathbf{u}(t)$ over a tidal cycle, residual current vectors ($\rightarrow$) tangential to which a residual streamline $\mathcal{L}$ is drawn. The instantaneous velocity vector can be decomposed into a component $\mathbf{u}_{\parallel}$ tangential to $\mathcal{L}$ and a component $\mathbf{u}_{\perp}$ perpendicular to $\mathcal{L}$.

Vorticity dynamics

The relevance of vorticity to residual eddy dynamics can readily be appreciated if the concept of circulation, which can easily be visualized and has a close connection with vorticity, is introduced as a measure of eddy strength.

Figure 2 shows a hypothetical string of current velocity meters in a quasi two-dimensional (horizontal) tidal velocity field. A simple time-dependent velocity field is assumed. At each position the velocity has two components: one which is strictly periodic in one of the basic tidal frequencies, σ , and the other is a time-independent residual. The current meters are supposed to produce non-vanishing residual-velocity vectors (tidal averages), tangent to which a curve $\mathcal{L}$ is drawn. The curve itself represents a residual streamline; its shape suggests the presence of a residual eddy in the field. The instantaneous velocity vector at each point of $\mathcal{L}$ is

$$\mathbf{u}(t) = \mathbf{u}_{\parallel}(t) + \mathbf{u}_{\perp}(t) \quad (1)$$

where $\mathbf{u}_{\parallel}$ and $\mathbf{u}_{\perp}$ are components tangential and perpendicular to $\mathcal{L}$ respectively. Denoting tidal averaging by $\sim$, we have by definition of $\mathcal{L}$ and in view of the simple time dependence of the velocity field:

$$\begin{aligned} \tilde{\mathbf{u}}_{\perp} &= 0, \quad \tilde{\mathbf{u}}_{\parallel} = \tilde{\mathbf{u}} \\ \mathbf{u}_{\perp} &= \mathbf{u}'_{\perp} \sin(\sigma t + \phi_{\perp}), \quad \mathbf{u}_{\parallel} = \tilde{\mathbf{u}}_{\parallel} + \mathbf{u}'_{\parallel} \sin(\sigma t + \phi_{\parallel}) \end{aligned} \quad (2)$$

where $\phi_{\perp}$ and $\phi_{\parallel}$ are arbitrary phase angles. To get some measure of the eddy strength, the circulation, C , around $\mathcal{L}$ is now defined by the contour integral:

$$C(t) = \oint \mathbf{u}(t) \cdot d\mathbf{l} = \oint u_{\parallel}(t) dl \quad (3)$$

By Stokes' theorem this integral equals an integral over the surface A enclosed by $\mathcal{L}$:

$$C(t) = \iint_A \omega dA \quad (4)$$

where ω , the vorticity of the current velocity field, can be interpreted as the 'spin' or twice the angular velocity around a

vertical axis of an infinitesimal fluid element inside $\mathcal{L}$. Now, taking tidal averages, the residual circulation reads

$$\tilde{C} = \oint \tilde{u}_{\parallel} dl = \iint_A \tilde{\omega} dA \quad (5)$$

Thus, if residual circulation around a closed curve in a tidal area is positive (anticlockwise or cyclonal) or negative (clockwise or anticyclonal) then, on average, a distribution of negative or positive residual vorticity ('spin') of the fluid elements inside the area exists over the area enclosed by the curve.

To find when tidal regions exhibit areas of non-vanishing tidal average vorticity we need to look more closely at the equation of motion of a quasi two-dimensional homogeneous fluid flow in a rotating frame of reference:

$$\frac{\partial \mathbf{u}}{\partial t} = -\{f + \omega\}(\mathbf{j} \times \mathbf{u}) + \nabla(g\zeta + u^2/2)$$

local acceleration =

Coriolis + vortex force + dynamic pressure gradient (6)

$$-\frac{r}{D} \mathbf{u} + \nu \nabla^2 \mathbf{u}$$

(linearized) bottom friction + horizontal turbulent momentum exchange

The local acceleration at a particular position in the field equals the sum of the forces in the right-hand side of equation (6); f denoting the Coriolis parameter (the planetary vorticity); g , the acceleration of gravity; ζ , the departure of the sea surface from its mean position; r , a bottom friction parameter; D , the water depth; and ν , a turbulent viscosity coefficient. Neglecting the last term in equation (6) the equation of motion is readily transformed into an equation for the circulation around $\mathcal{L}$, which reads

$$\frac{\partial C}{\partial t} = \oint (f + \omega) u_{\perp} dl - \frac{r}{D} C \quad (7)$$

The residual circulation is obtained by averaging equation (7) over a tidal cycle:

$$\begin{aligned} \tilde{C} &= \frac{\oint \overline{\omega(t) u_{\perp}(t)} dl}{r/D} = \\ &= \text{tidal mean flux of vorticity through } \mathcal{L} \\ &\quad \times \text{relaxation time scale of bottom friction} \end{aligned} \quad (8)$$

Thus a non-zero (residual) circulation around $\mathcal{L}$ is produced if, when averaged over the tidal cycle, there is a net flux of vorticity into the region enclosed by the curve (a net outflux is equivalent to a net influx of vorticity of opposite sign). Such 'pumping' leads to the build-up of positive or negative residual vorticity, ultimately balanced by the dissipation due to bottom friction, or, had we included that effect in equation (7), to horizontal turbulent vorticity diffusion. Note that $\overline{\omega u_{\perp}} dl$, having dimensions $[L^2 T^{-2}]$, can be interpreted as a stress (momentum flux), often called the 'tidal stress'^{5,6}. Hence the residual vorticity flux can be thought of as the analogue of a time-independent tangential stress exerted along the boundary of the water column inside $\mathcal{L}$, which therefore tends to spin-up.

For a non-vanishing residual circulation we need to both produce vorticity somewhere in the field and achieve a net flux of vorticity through a closed curve over a tidal cycle. Thus, whereas the condition necessary for residual circulation, expressed by equation (8), is now revealed physically, how does such a net flux arise? Mathematically, if $u_{\perp} \propto \sin(\sigma t + \phi_{\perp})$ and $\omega(t) \propto \sin(\sigma t + \psi)$, it is the phase difference $\phi_{\perp} - \psi$ that determines sign and magnitude of the pumping term $\overline{\omega u_{\perp}}$. If $\phi_{\perp} - \psi$ is near to 0 or π that is if $u_{\perp}$ and ω change sign approximately at the same moment in the tidal cycle, then conditions are favourable for the

Table 1 Observed orders of magnitude of residual vorticity

Area	Interpretation measurements supported by	Eddy characteristics			Tidal velocity amplitude (m s ⁻¹)	Eddy type	Refs
		Velocity (m s ⁻¹)	Length scale (10 ³ m)	Vorticity (s ⁻¹)			
a Current measurements							
Kasado Bay Japan	Numerical ⁸ /hydraulic ^{7,19} model	0.1	2	5 × 10 ⁻⁵	4.10 ⁻¹	a	44
Minas Basin Bay of Fundy	Numerical ¹⁰ model	0.2	8	3 × 10 ⁻⁵	1.5	b	45
Portland Peninsula English Channel	Numerical model	0.3	30	10 ⁻⁵	?	b	11, 21
Norfolk sand banks North Sea	Analytical model/bottom topography	0.1	30	3 × 10 ⁻⁶	1?	c	13
Wadden Sea	Bottom topography	0.1	5	2 × 10 ⁻⁵	0.9	a?/c	24, 33
Cook Inlet Alaska		0.2	10	2 × 10 ⁻⁵	1.5	c?	17
Southern Bight North Sea	Analytical model/bottom topography	0.01	40	10 ⁻⁷ –10 ⁻⁶	0.7	c	18
Prototype							
b Numerical models							
Kasado Bay		0.01	5 × 10 ⁻³	2 × 10 ⁻³ (10 ^{-5*})	5.10 ⁻³	a	8
Start Point Promontory		0.3	4	<10 ⁻⁴	1	b	12
English Channel		0.2	20	<10 ⁻⁵	1	b	22
Southern Bight North Sea		0.05	15	3 × 10 ⁻⁶	1	c?	5
Southern Bight North Sea		0.1	20	5 × 10 ⁻⁶	0.7	c?	23
c Hydraulic models							
Kasado Bay		0.01	5 × 10 ⁻³	2 × 10 ⁻³ (10 ^{-5*})	5.10 ⁻³	a	7,19
Seto Inland Sea		0.15*	20*	10 ^{-5*}	1	b	6,20

Values are overall estimates, subject to considerable uncertainty.

* Model result rescaled to the prototype; eddy types are in accordance with Fig. 3.

buildup of residual vorticity. Figure 3 indicates when such favourable conditions are met, summarizing the different situations dealt with recently. We classify these situations according to the geomorphological background.

(1) Residual basin circulation (Fig. 3a, refs 7–9) is the simplest example of tidal residual circulation and is given by the distribution of ebb and flood surpluses, generated by frictional boundary layers in a semi-enclosed basin. In such a basin the tidal-current velocity diminishes both towards the back wall and, by friction, towards the side walls. By the latter process vorticity is generated, the sign of which is opposite at both sides of the basin and changes with the current direction. As the tidal current decreases towards the back wall, at the left-hand side of the basin, during the flood, more anticlockwise vorticity is brought into the heavily drawn curves near the entrance than is transported outwards near the back wall. During the ebb more clockwise vorticity is brought out, which is equivalent to an influx of anticlockwise vorticity. Thus, averaged over the tidal cycle there is a net influx of anticlockwise vorticity resulting in an anticlockwise residual circulation along the heavily drawn curve in the left-hand side of the basin. The opposite occurs on the right-hand side where a clockwise residual circulation is set up. Thus a flood surplus exists in the middle of the basin and an ebb surplus along the sides.

(2) Headland eddies (Fig. 3b; refs 10–12)—along a coastal promontory the tidal flow is accelerated and decelerated in the current direction and has a maximum near the headland. Concurrently a frictional boundary layer develops diminishing the current velocity towards the coast. Thus vorticity is produced along the coast with its sign alternating between the ebb and flood stages of the tide. As the vorticity is largest near the headland there is a net flux of clockwise vorticity into the upper heavily drawn curve and out of the lower one when the current direction is upwards, during the flood, for example. During the ebb, the situation does not reverse because of a net flux of anticlockwise vorticity out of the upper curve and into the lower curve, which is equivalent to the situation during the flood. Hence there is a net flux of clockwise vorticity into the upper curve and of anticlockwise vorticity into the lower curve, giving rise to a vortex pair of opposite sign at both sides of the promontory.

Note that by placing at the left-hand side of Fig. 3b its mirror image, a quadruple vortex system is obtained which represents

the situation close to a sudden narrowing of a tidal channel, as often occurs near inlets connecting tidal channels and the open sea.

(3) Sand ridge eddies (Fig. 3c/d; refs 13–16)—when a tidal current crosses a submarine sand ridge, the flow is accelerated going up the slope and decelerated going down the slope. Consequently a water column experiences a slightly higher Coriolis force on its shallower side than on its deeper side. Thus a 'Coriolis torque' exists on the column having opposite sign at both sides of the ridge, and, at the same side, at both stages of the tidal period. A torque is also produced by bottom friction, the latter being stronger at the shallower side of a fluid column. Both torques reinforce each other if the ridge is inclined anticlockwise with respect to the tidal current (Fig. 3c), whereas they counteract in the opposite case (Fig. 3d). Taking a curve around the ridge, it is obvious that in case (c) there is a net influx of clockwise vorticity or, equivalently, an outflux of anticlockwise vorticity produced by both torques during the flood (current velocity upwards), assuming that vorticity production is quasi-static, that is the instantaneous sign of vorticity is the same as that of the applied torque. The same occurs during the ebb (current velocity downwards), so that a residual influx of clockwise vorticity produces a clockwise residual circulation around the ridge. In case (d) the same applies to residual circulation related to vorticity production by Coriolis torques but the reverse applies to the residual circulation produced by bottom frictional torques, the latter now giving rise to anticlockwise residual circulation. As both mechanisms counteract, a weaker net circulation may be expected compared with case (c). Of course, this applies to situations in the Northern Hemisphere. In the Southern Hemisphere ridges inclined clockwise with respect to the tidal current would be expected to show reinforcing of both mechanisms.

Scale effects

Are residual eddies of all sizes equally probable or are eddies of a specific horizontal extent preferred dynamically? This question, relevant to the geomorphology of the sea bed, has only previously been answered^{14,15} for the production mechanisms shown in Fig. 3c, d, that is residual eddies produced by depth gradients in the open sea. Consider a test topography (Fig. 4a) consisting of a regular series of humps and bumps with a height or depth h below the mean depth H and a horizontal wavelength

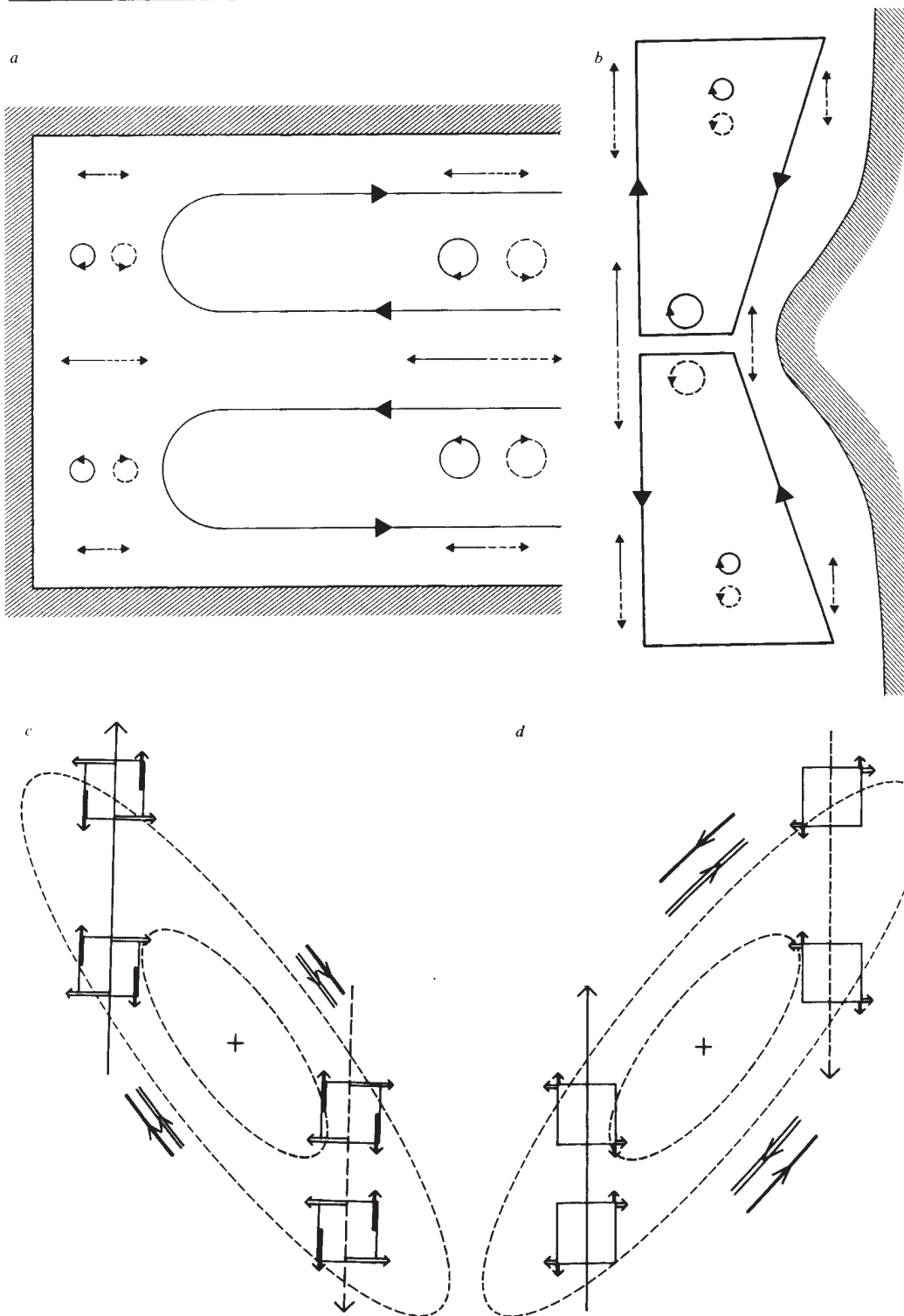


Fig. 3 The basic production mechanisms of residual circulation in tidal currents through sidewall friction (a, b) or through Coriolis and bottom frictional torques (c, d). a, Rectilinear tidal currents in a semi-enclosed basin are shown by solid arrows for the flood period and dashed arrows for the ebb period. The currents decrease both towards the end of the basin and towards the side walls. Vorticity generated by side-wall friction is shown by solid circles for the flood period and dashed circles for the ebb period. The vorticity has opposite signs at both sides of the basin and diminishes towards the back wall. The resulting residual circulation is shown by heavy streamlines. b, Tidal currents, tidal vorticity and residual circulation in the neighbourhood of a headland promontory have the same symbols as in a. Tidal currents are largest near the headland and decrease towards the coast. Tidal vorticity therefore has a maximum near the headland. c, d, A submarine sand ridge is shown by ellipsoidal dashed depth contours; + represents the top of the ridge. Rectilinear tidal currents, solid arrows for the flood period, dashed arrows for the ebb period, cross the ridge, which in c is inclined anticlockwise with respect to the current and clockwise in d. By the presence of bottom depth gradients both the Coriolis force and bottom friction produce torques at a fluid column as shown by the open arrows for the former and solid arrows for the latter. The situation during the flood period is only shown for the left-hand side of the ridge and during the ebb period only for the right-hand side. The bottom frictional torques change sign if the inclination of the ridge changes from anticlockwise to clockwise, whereas the Coriolis torques remain unchanged. The resulting residual circulation around the ridge is shown by solid and open arrows, parallel to the depth contours, the arrows denoting respectively circulation produced by the bottom friction mechanism and by the Coriolis mechanism.

L . The tidal current is characterized by a velocity amplitude U and the frequency σ , which produce a length scale, the amplitude of horizontal tidal excursion, l :

$$l = U/\sigma \quad (9)$$

Together, tide and topography are now characterized by two non-dimensional parameters, a relative depth h/H and a wavenumber, λ :

$$\lambda = \frac{2\pi l}{L} = \frac{\pi \times \text{tidal excursion}}{\text{topographic wavelength}} \quad (10)$$

The ratio ν of the residual speed around a curve enclosing a

hump and the amplitude of tidal velocity is^{14,15}:

$$\nu = \frac{\bar{u}}{U} = \frac{h}{H} g(\lambda) \quad (11)$$

The weighting function $g(\lambda)$ is shown in Fig. 4b for both vorticity production mechanisms—Coriolis and bottom frictional torques. An interval of length scale ratios centred around $\lambda \approx 1$ leads to a strong resonance in terms of residual tidal velocity. The latter falls off rather rapidly for both smaller and larger length scale ratios. This is because for very large topographic length scales vorticity gradients in the field vanish, so that no net flux of vorticity into a closed curve can be produced. For very

small length scales fluid columns experience during the tidal cycle such a rapid sign reversal of the vorticity production mechanisms that effectively no vorticity can be built up, so that no vorticity can be transferred to the mean field. The 'resonance peak' is reflected in the asymptotic behaviour¹⁵ of $g(\lambda)$:

$$\begin{aligned} g(\lambda) &\propto \lambda, & \lambda \rightarrow 0 \\ g(\lambda) &\propto \lambda^{-1}, & \lambda \rightarrow \infty \end{aligned} \quad (12)$$

Hence, in view of the definition of λ ,

$$\begin{aligned} \tilde{u} &\propto U^2 \frac{h}{H} L^{-1}, & \lambda \rightarrow 0 \\ \tilde{u} &\propto \frac{h}{H} L, & \lambda \rightarrow \infty \end{aligned} \quad (13)$$

Thus, whereas the residual velocity increases with the square of the tidal velocity amplitude for large topographic length scales, it becomes independent of U for smaller length scales.

Observations and model studies

Very few direct measurements of residual eddies exclusively from current velocity data exist; the evidence is usually indirect in that current velocity observations of eddy-like structures are complemented by knowledge about the bottom morphology or coastal geometry, or by modelling of the flow field. This particularly applies to the spatial structure of the eddies where, for complete spatial coverage in a rather small area, a very large array of instruments would have to be used simultaneously. The former difficulty can in principle be overcome by a new method of surface current measurements using Bragg scattering of HF-radar rays, used for the observation of surface wind waves. The surface current component in the radial direction from the antenna can be determined by observing the Doppler shift in the phase velocity of the waves. By using two antennas some distance apart the complete velocity vector can be measured. Hence an area of $\sim 2,500 \text{ km}^2$ can be mapped with a high resolution in time and space, allowing residual currents to be detected by averaging the observed velocity field in time over the dominant tidal period(s).¹⁷ At present, however, its accuracy, $\sim \pm 10 \text{ cm s}^{-1}$ for an individual current speed determination, means that the method is only successful for residual currents if the associated tidal currents exceed a value of 1 m s^{-1} , say, as the residuals are usually $< 10\%$ of the tidal currents proper.

Important parameter values of residual eddies are shown in Table 1, where the crucial parameter is the residual vorticity, which can vary over some orders of magnitude, from 10^{-4} to 10^{-7} s^{-1} , generally decreasing from the coastal zone to the open sea. It never seems to be larger than the so-called planetary vorticity (the Coriolis parameter), f , which at mid-latitudes is of the order of 10^{-4} s^{-1} . This may just be coincidence for basin or headland eddies (Fig. 3a, b), but can easily be explained for sand-ridge eddies (Fig. 3c, d) where the Coriolis force has an important role. In that case the residual vorticity is, in fact, derived from the planetary vorticity by vortex stretching and thus is unlikely to be larger than it. Typical associated velocities lie between 1 and 10 cm s^{-1} and length scales between 3 and 30 km .

In contrast to the eddy properties inferred from current measurements in the sea, results obtained exclusively from numerical or hydraulic modelling should be interpreted with care, as the appearance of eddies in these models depends critically on the basic equations, the boundary conditions and the grid size used in a numerical model and on the scaling used in a hydraulic model.

Analytical theories tend to use highly simplified geometries such as a rectangular basin⁹, a linearly sloping bottom¹³ or a bottom consisting of a superposition of plane horizontal waves^{14,15,18}. Although these approximations contain the basic physics, simulating the tidal velocity field, in particular the residual field, would enable us to cope with complicated situations that cannot be handled analytically. This can be done either by direct simulation in a hydraulic scale model or by

numerically solving the nonlinear equation of motion, (6), on a grid covering the investigated area, for some suitably chosen boundary conditions.

Successful hydraulic scale modelling depends critically on whether all dominant terms in the basic equation are scaled in the same proportion (similitude). If not, the flow field of the model is likely to be distorted compared with the field in the prototype. For residual eddies, only hydraulic models for the side-wall friction mechanism (Fig. 3a, b), not incorporating rotational effects, have been described for an enclosed basin^{7,19} and for eddies produced by headlands or islands in the Seto Sea (Japan)^{6,20}. Similitude may be expected if the Reynolds number, R_e ,

$$R_e = \frac{UL_b}{\nu} \frac{L_b}{L} \quad (14)$$

based on the ratio of vorticity advection and vorticity diffusion, is the same in model and prototype; U being the tidal velocity amplitude, L the headland or basin dimension, ν the horizontal turbulent viscosity and L_b the thickness of the frictional boundary layer. Because $L_b \propto (\nu/\sigma)^{1/2}$, where σ is the tidal frequency, this leads to similitude of

$$R_e = \frac{U}{\sigma L} \frac{\text{tidal excursion}}{\text{basin or headland length scale}} = \lambda \quad (15)$$

Again, the ratio between tidal excursion and topographic length scale is the basic dynamical parameter. The influence of the Earth's rotation makes hydraulic simulation much more complicated for the mechanisms displayed in Fig. 3c, d because here, as in the prototype, the model should rotate. No results have yet been reported.

Numerical modelling results are only known^{8,10-12,21,22} for situations which belong essentially to the mechanisms of Fig. 3a, b. Side-wall friction is sometimes effectively produced by introducing a bottom sloping upwards to the coast, otherwise a no-slip condition at the boundary is applied. Anyhow, decreasing velocity towards the side-walls is crucial in this case because the absence of such velocity gradients means that no vorticity is present in the velocity field, so that no residual circulation can be built up. Along the boundaries these gradients are either

Table 2 Inventory of linear tidal sand ridges according to ref. 4, together with tidal current velocities and length scale ratios λ , assuming that tides are predominantly of the semidiurnal lunar type (M_2)

Area	Average ridge length (10^3 m)	Average tidal current velocity amplitude (m s^{-1})	λ
Great Bahama Bank	22.5	0.75	1.5
Rio Amazon	54.0	2.5	2.1
Sao Luiz do Maranhao (Brazil)	13.5	1.2	5.0
Malacca Strait	31.5	1.5	2.0
River Gambia to River Jeba (Africa)	15.0	0.75	1.3
Yalu River to Taech'ong Kudo	72.0	1.0	0.6
Wa Chiu Hsu to Tung-yin Shan	67.5	0.5	0.3
Keppel Isles to Whitsunday Island (Australia)	18.0	1.0	2.5
Prince of Wales Channel	13.5	0.5	1.7
	13.5	1.0	3.3
Persian Gulf	54.0	0.5	0.3
	54.0	1.0	0.3
	13.5	1.1	3.8
Southern Bight North Sea	49.5	0.7	0.6
Dover and Calais	27.0	1.1	1.8
	21.0	0.8	1.7
	21.0	0.8	1.7
	14.5	0.8	2.5
Rio Guayas Ecuador	13.5	1?	3.3?
Bahia Blanca Argentina	12.0	0.7	2.4
	12.0	0.5	1.9
Gulf of Maine/Georges Bank	36.0	1.0	1.3
Kuskokwim Bay, Alaska	27.0	0.9	1.5
	31.5	0.8	1.1
Nyshagak Bay, Alaska	18.0	1.2	2.9
Overall averages and standard deviations	29.0 ± 19	0.9 ± 0.4	1.9 ± 1.2

supplied by viscous friction at the wall or by differential bottom friction over a bottom sloping perpendicular to the wall. Hence a no-slip condition is essential when bottom slopes near the wall are absent or weak¹⁰. This condition is often not met in numerical modelling of tidal currents. Such models therefore do not produce residual eddies by the side-wall frictional mechanism, though they may be present in the prototype. Numerically it is not difficult to keep the Coriolis acceleration in the model, but its effect on eddies produced by side-wall friction seems to be minor¹⁰.

Finally, although there are no specific results from numerical modelling of the bottom slope mechanisms (Fig. 3c, d), some of the residual eddies appearing in tidal models, such as those of the North Sea^{5,23}, should be ascribed to these mechanisms. However, the grid size of these models is generally much larger than the amplitude of the tidal excursion. Thus, the shape of the weighting function shown in Fig. 4b suggests that the optimum topographic length scales for production of residual circulation are not resolved by such models. Residual circulation then only appears if the bottom topography fluctuates strongly on larger length scales and, in fact, it is in such a case that the models mentioned show residual circulation. Hence much of the fine structure in the residual field may be suppressed by choosing too large a grid size. This indicates that the curves in Fig. 4b imply a criterion for the set-up of a suitable grid for the numerical calculation of residual circulation¹⁴, provided the bottom topography is properly reproduced on all length scales from the grid scale upwards. Residual circulation induced by the topography can be modelled successfully if the grid size is of the order of, for example, half the tidal displacement, in which case most of the responsive scales in terms of residual velocity are covered. A much smaller grid would be more time consuming in the computation without showing much more structure in the residual velocity field. On the other hand, a larger grid size does not resolve the optimum scales where eddies may be found.

Residual eddy diffusion

Tidal currents carry waterparcels only to and fro or around in a closed orbit. No waterparcel can escape these predetermined pathways unless some other velocity component allows a jump from one tidal pathway to another. If these jumps occur randomly, the rate of change of the spread of an ensemble of waterparcels can be used to define an effective diffusion coefficient. Normally, in a turbulent fluid, such a coefficient can be expressed in the velocity and length scales of the largest turbulent eddies present in the flow. This, of course, also applies to turbulent tidal flow regimes, where residual eddies constitute the largest scales, though it is not *a priori* clear that such eddies diffuse matter in the same way as real turbulence does. Nevertheless, it is tempting to assume that the horizontal 'residual eddy diffusion coefficient', K_e , can be expressed as

$$K_e = c\tilde{u}L \quad (16)$$

where $\tilde{u}$ and L are characteristic residual velocity and length scales. This expression has little use unless the dimensionless constant, c , can be estimated. For two areas, the Seto Inland Sea in Japan⁶ and the Dutch Wadden Sea^{16,24,25}, this could be achieved by estimating K_e from the salinity budget of the area and, independently, by estimating characteristic values for $\tilde{u}$ and L from the current velocity field. For the Seto Inland Sea c appeared to be of the order of 0.4–0.5, whereas for the Dutch Wadden Sea values between 0.5 and 2.0 were obtained, compatible with diffusion coefficients of the order of 1,500 m² s⁻¹ and 400–1,500 m² s⁻¹ respectively. Thus, if equation (16) is a correct expression for K_e , c seems not to be a real constant, but must depend on (an) additional parameter(s). A guess^{16,24,26} is that these parameters are the velocity and length scale ratios λ and ν encountered previously (equations (10) and (11)). Different values of c may then be explained by these ratios (particularly λ) varying between areas. Table 1 shows that representative ranges of ν and λ are 0.1–0.2 and 0.5–3.0 respectively. The few theories^{16,24,26} which explain the depen-

dence of c on ν and λ then give a range of 0.2–2.0 for c , which is of the same order as of the values derived empirically from the salinity budget in the case studies mentioned previously.

Geomorphological implications

The shape of the coastal geometry or the bottom morphology seems to be important to any tidal residual eddy theory, but is there also a reverse effect of the residual eddies on the morphology; does any specific shape of the morphology depend on the characteristics of residual eddy circulation? Such a connection is most obvious for eddies of the type shown in Fig. 3c, d. The bottom morphological structures involved are the so-called 'linear tidal sand ridges', the worldwide appearance of which has been reviewed in ref. 4. Table 2 contains information about tidal velocity amplitudes for the areas discussed in ref. 4 together with estimates of the length scale ratio λ —the fundamental parameter in residual eddy dynamics. The average length of the sand ridges is 29 ± 18 km, whereas the average tidal velocity amplitude is 0.9 ± 0.4 m s⁻¹. More relevant is the average value and spread of λ , which reads 1.9 ± 1.2 . As Fig. 4b shows this is exactly the range where maximum response of residual circulation can be expected. More recent studies^{27–29} of areas not included in ref. 4 show the same order of magnitude for the length of the ridges relative to the tidal excursion amplitude. The observation that the value of λ , on average, coincides with that for maximum response of residual circulation around the ridges, suggests that during their formation the ridges somehow adjust their shape so as to maintain the largest possible residual velocities around their contours. This suggestion is intensified by the observation^{29,30} that linear sand ridges are not exactly aligned along the major axis of the tidal current ellipse, but are offset in an anticlockwise direction (8–15°) as far as ridges on the Northern Hemisphere are concerned (no observations are at hand of ridges on the Southern Hemisphere but these must show the reverse effect, clockwise inclination). As shown in Fig. 3c, d

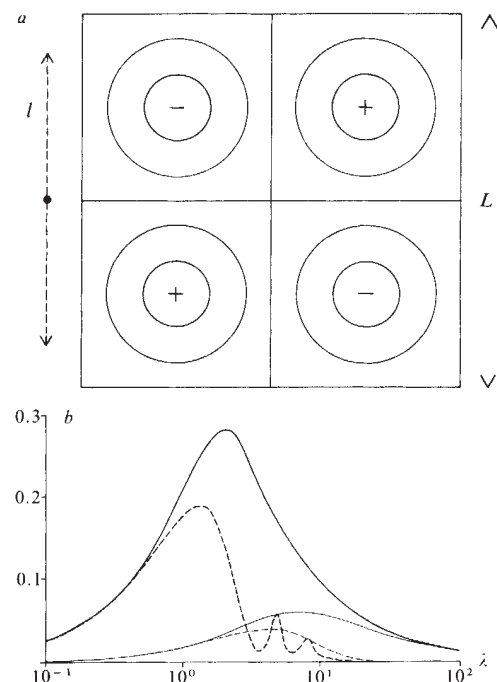


Fig. 4 a, A test topography consisting of a regular series of humbs and bumps of height (+) or depth (-) below the mean depth H . The wavelength L should be compared with the amplitude of tidal excursion l . The weighting function $g(\lambda)$ (refs 14, 15) for the Coriolis effect (solid curves) and for the bottom frictional effect (dashed curves), both for two values of the inverse bottom friction time scale r/H in equation (6); $r/H = 10^{-4}$ s⁻¹ (the highest solid and dashed curves) and $r/H = 10^{-3}$ s⁻¹ (the lowest solid and dashed curves). Note that for both vorticity production mechanisms $g(\lambda)$ has a maximum for λ in the range 1–5. The position of the maximum shifts to higher values of λ for increasing bottom friction coefficient or, equivalently, for decreasing mean water depth.

the ridges inclined anticlockwise with respect to the tidal current direction produce the largest response of residual circulation, as in that case bottom friction is reinforcing the Coriolis effect in setting-up a clockwise circulation around the bank. The geomorphological evidence shows, therefore, that both length scale and inclination of the ridges relative to the tidal current are generally such that maximum residual circulation around the ridge is produced, suggesting that this is necessary to maintain the ridge shape, which otherwise might degrade in the presence of other flattening or erosive processes. This may have some bearing on the interpretation of palaeo-environments, as these ridges are also observed in areas where a strong tidal influence no longer exists or where such ridges are buried under more recent sediments^{29,31}. For instance, the relict linear ridges found north-west of the Dogger Bank in the North Sea³¹ are thought to have been formed at ~7,000 BC when the sea level was 40–45 m lower. Whereas tidal currents are running north–south presently at the site, with a speed of $\sim 0.5 \text{ m s}^{-1}$, the orientation of the ridges implies that, at the time of their active formation, currents were predominantly running (northeast–south)west, more or less parallel to a shoreline being formed by the northern flank of the Dogger Bank. According to the length of these relict ridges, on average 33 km (ref. 31), tidal current speeds must have been at least twice as high as they are there now.

In the case of headland eddies (Fig. 3b) it is less clear that the headland geometry itself is affected by the tidal current regime: in fact, nontidal factors principally determine the shape of the coastline. However, once the headland exists, it may produce a residual vortex pair. These vortices, in turn, collect sediment, thereby leading to the formation of sand banks^{11,12,32}. If centrifugal and Coriolis forces are both of equal importance in the eddies, sediment may accumulate asymmetrically between the eddies forming a vortex pair. The eddy showing anticlockwise circulation is preferred (on the Northern Hemisphere), because then Coriolis and centrifugal forces together with bottom friction reinforce each other in producing a convergence of bottom currents to the centre of the eddy¹¹. Thus, whereas the principal residual eddies do not depend on the existence of sand banks but on the headland geometry, imbedded within these principal eddies may be smaller ones coupled to the sand banks, which in turn are due to the sediment concentrating capacity of the principal eddies.

Finally, the geomorphological significance of the basin eddy in Fig. 3a is uncertain because it contradicts observations that in tidal channels the flood normally exceeds the ebb on the shallow sides of the channel, whereas the centre of the channel is ebb dominated^{33–36}. The sides, however, are often formed by drying tidal flats and there has been no explanation for the peculiar effects that may arise from areas uncovered by water for some time during the tidal cycle. The meandering of ebb-dominated gullies can set up a system of smaller residual cells within the overall residual pattern^{35,37,38}. It remains to be seen whether the dimensions of these cells are within the range of maximum

response for bottom morphological eddies; an observation from the Dutch Wadden Sea indicates that they may²⁴. In contrast to more open-sea areas the residual eddies suggested by ebb and flood channels in these shallow inland waters have a smaller dimension compared with the tidal excursion (a larger λ). This agrees with the result that a decrease of the bottom friction time scale, which is equivalent to a decrease in water depth, shifts the resonance peak to higher values of λ , as can be seen in Fig. 4b.

Conclusion

The existence of residual eddies in tidal areas can be largely explained by quasi two-dimensional vorticity dynamics together with a coastal geometry and/or bottom morphology providing tidal vorticity gradients. This theory does not really explain the characteristics of bottom morphological structures from the residual current velocity pattern, however. First, in residual sediment transport not only residual currents should be taken into account but also the nonlinearly generated higher harmonics in the tidal velocity field, the so-called overtides^{12,39,40}. Yet, if sediment transport can be expressed as a function of the vertically averaged velocity, this may not be a serious flaw of quasi two-dimensional theory as overtides appear as a by-product of residual current theory^{12–15}. Hence, a two-dimensional approach requires incorporation into the theory of a description of the interaction between (time-varying) sea-bed morphology and the tidal current regime. Such a theory might also give a first explanation of whether the bottom morphology is static or whether it may shift in space over a long time as is sometimes observed^{27,41}. However, the dimensionality of the present theories is a severe restriction. For problems of suspended sediment transport, the tidally varying vertical distribution of suspended load and velocity is important in producing residual transport, whereas for transport as bed-load the direction of the bottom current is decisive. In particular, the veering of the current direction vertically determines whether bottom currents, carrying the bulk of the sediment load, converge or diverge around any pre-existing bottom structure. Linear sand ridges, for instance, can only be maintained if there is a convergence of sediments towards the ridge crest, as is also observed indirectly by the inferred direction of bed-load transport from the properties of small sand waves on the sides of the ridge³⁰. Observational evidence on veering of tidal and residual currents near sand ridges is scarce, although the few observations we have^{42,43} support the conclusion derived from the sedimentary record³⁰. However, such observations cannot be interpreted in terms of our two-dimensional theories, and we lack the relevant three-dimensional theory.

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ARTICLES

Photoelectron spectromicroscopy

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Photoelectron spectroscopy is a powerful technique for studying electronic structure both of isolated molecules in the gas phase and of surfaces and adsorbed layers. Surfaces have also been imaged using photoemission microscopy. A new technique is described for combining the features of position imaging and energy analysis of the photoemitted electrons. The imaging is achieved using image projection in a divergent magnetic field to preserve the electron energy distribution.

DURING the construction of an instrument for the study of ions trapped in a high magnetic field we have explored other possibilities which this unusual arrangement of a magnetically collimated photoelectron energy analyser offers. In particular we considered imaging a surface using energy-analysed electrons. The redistribution between transverse and axial energy has a central role which depends on the divergence of the magnetic field, and this divergence produces magnification. Thus microscopy using energy selected photoelectrons is a possibility¹.

Energy analysing photoelectron microscopy

The new technique using photoelectron emission which may be seen as an extension of earlier experiments with conventional-electron microscopes²⁻²², but introduces a new principle of electrostatic field-free imaging. The incident radiation (for

example, $\text{He(I}\alpha)$) has a much higher energy (21.2 eV) than the work function. This enriches the image contrast by a wide range of photoelectrons from different valence levels. Energy analysis of the photoelectrons then allows spectroscopic analysis of the image.

The new technique depends on the capability of an axially symmetric divergent magnetic field to generate the enlarged image of a photoemissive object while preserving the original total energy distribution. Because the orbital moment of an electron moving in a divergent magnetic field is conserved as well as its total energy, any initially transverse energy reappears as energy of axial motion¹.

$$E = E_{\text{total}} - E_{\text{cyclotron}} \frac{B(2)}{B(1)}$$

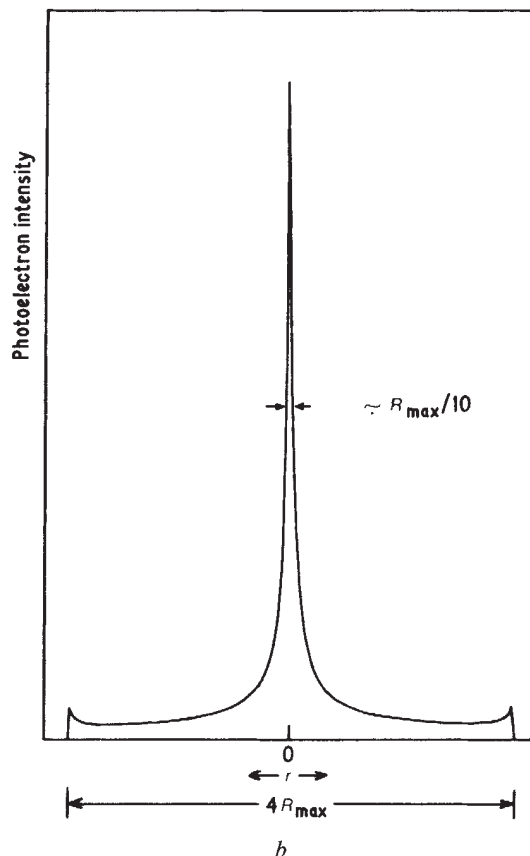
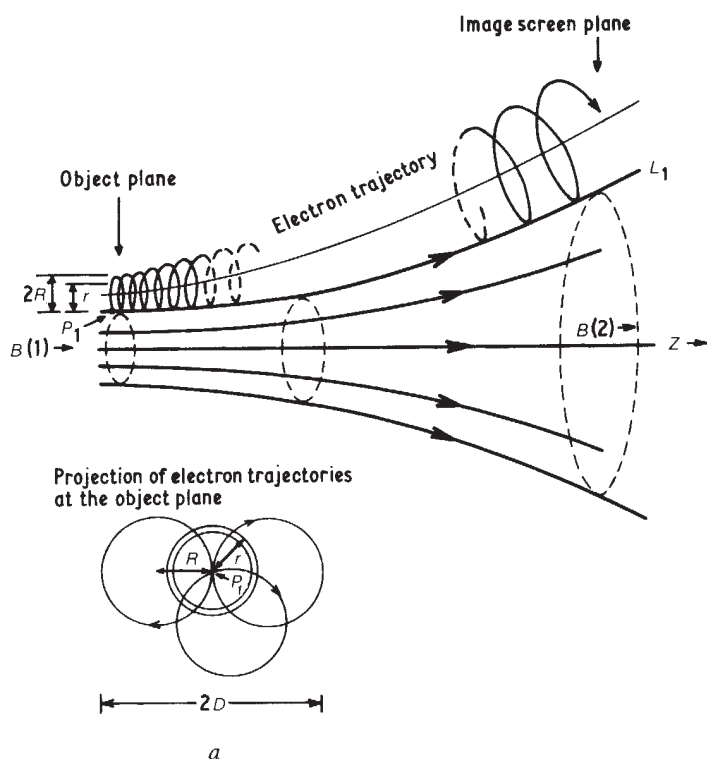


Fig. 1 *a*, Motion of a photoelectron in a divergent magnetic field. The electron is emitted at P_1 on the flux line L_1 . Inset, projection of trajectories at the object plane which determine a maximum circle of confusion (see text). *b*, The calculated intensity of photoelectron flux across the image of a point source for $\beta = 0$. The dimension $(4R_{\text{max}})$ is determined by the largest possible orbit radius R_{max} at the object surface for a given value of magnetic field and total electron energy (see text).

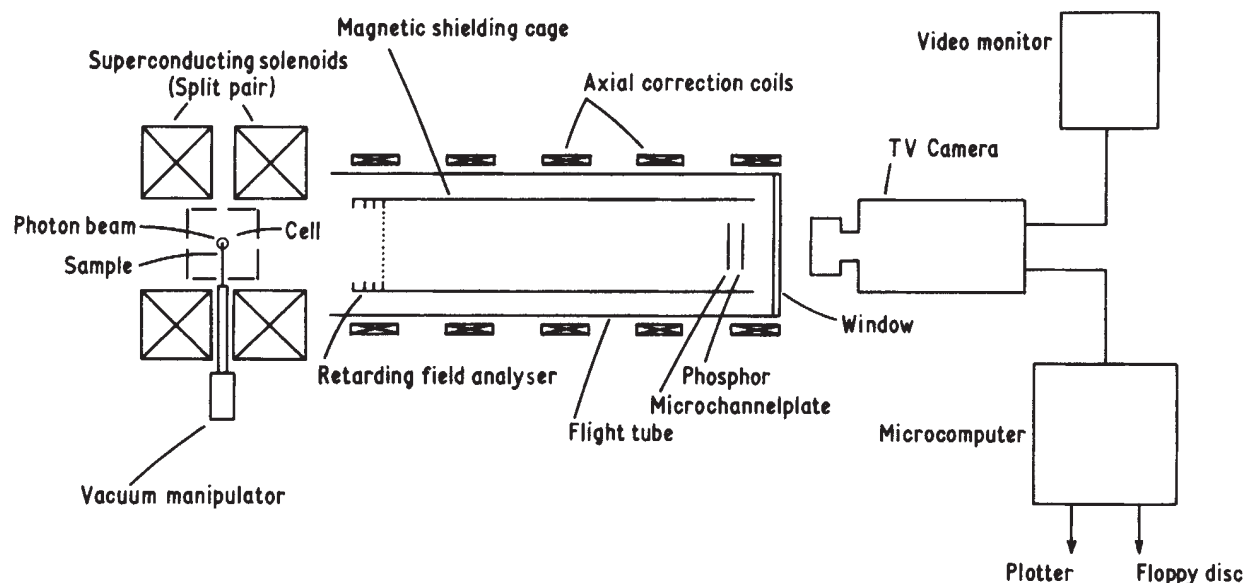


Fig. 2 The magnetic projection photoelectron microscope.

where E is the final energy of forward motion, E_{total} is the total electron energy, $E_{\text{cyclotron}}$ is the amount of energy initially in cyclotron motion and $B(2)/B(1)$ is the field ratio (Fig. 1a). As no focusing elements are used, this photoelectron microscope has the maximum possible luminosity. It is a magnetic analogue of Müller's "point projection electron microscope". It is, however, capable of bringing to an image screen every photoelectron liberated with a non-zero Z -component of velocity with exactly its original energy.

The important features of the motion of an electron which originates from an object point P_1 in a high magnetic field (Fig. 1a) are, first, that the helical motion about the adjacent flux line adjusts itself to conserve the orbital moment as the flux lines diverge, so that the orbit radius is proportional to the magnification, the area magnification is $B(1)/B(2)$ and the lateral magnification is $(B(1)/B(2))^{1/2}$. Then, second and as a consequence, after a sufficiently large change of field the original photoelectron energy is concentrated in the axial motion. The first inference implies that the electrons return repeatedly to the flux line passing through the object point P_1 ; the second, that planar field retarding potential energy analysis can be performed on the total photoelectron flux at any convenient image plane in a low magnetic field region.

Lateral resolution

The resolution of such a photoelectron microscope will be limited (apart from any limitation imposed by the method used to observe the image) by deviation of an electron from the guiding field line, which is in turn determined by the original angular distribution of photoemission and the kinetic energy of the photoelectrons emerging from the surface of the object. The electrons emitted otherwise than parallel to the magnetic field execute a cyclotron orbit about the field lines and their original point of emergence is uncertain by a maximum of twice the maximum orbit diameter (D) at the surface of the object (see Fig. 1a). The cyclotron radius of an electron of total energy E_{total} emitted at an angle θ to the magnetic field direction is given by

$$R = \frac{(2mE_{\text{total}})^{1/2} \sin \theta}{eB}$$

where B is the magnetic field strength. The probability of an electron of cyclotron radius R being found within a radial distance r from the field line passing through its point of origin (Fig. 1a, inset) is given by

$$\frac{2}{\pi} \sin^{-1} \left(\frac{r}{2R} \right)$$

This probability must be weighted according to the original angular distribution of photoemission referred to the z -axis²³. The radial intensity distribution can be calculated assuming an appropriate value of β , the angular distribution parameter. Figure 1b shows the radial intensity distribution plotted for $\beta = 0$. Changes in β have little effect on the general shape of this function, which is dominated by the fact that all electrons must return repeatedly to the original field line L_1 . This function is so strongly weighted towards small r values, that we predict an ultimate effective lateral resolution (the diameter of a 'circle of confusion') of approximately $R_{\text{max}}/10$ arising from the cyclotron motion, where R_{max} is $(2mE_{\text{total}})^{1/2}/eB$.

Table 1 shows this expected lateral resolution for some useful electron energy and central (strong) magnetic field values, with the de Broglie wavelength of the free electron (λ_e) for comparison.

It is useful to compare the present method with other electron microscopies that provide chemical information. In electron probe microanalysis and scanning Auger electron microscopy, the lateral resolution is limited by the area of the probe electron beam at the surface and is typically 0.3–0.5 μm using a thermal cathode field emitting source. In scanning Auger microscopy with a cold-cathode field emitting source, the lateral resolution is limited²⁴ to ~ 30 nm and further improvement is prevented by electron scattering in the sample.

Thus from Table 1, in the present instrument, with a field strength of ~ 8 T, a useful microscopic performance is obtainable. For example, with 5-eV electrons, we could expect 0.1 μm resolution and in the highest readily attainable superconducting solenoid field, of ~ 16 T, performance should be at least comparable in lateral resolution to that of scanning Auger electron microscopy when lowest energy ($\approx kT$) photoelectrons are selected using samples at ordinary temperatures.

Depth resolution

Depth resolution (as opposed to lateral resolution) is sometimes referred to as depth of information and is related to the escape depth. (This should not be confused with depth of field, which is an instrumental property of a lens system and does not concern us because the present instrument is non-focusing; the field depth is limited only by the range of illumination of the object.) Thus a depth of information of d nm indicates that the final image is formed by photoelectrons that originate within a distance d of the surface.

The penetration depth of UV radiation is of the order of 1 μm but the electron escape depth is of the order of 0.5–20 nm. The precise value depends on the angle of exit and energy of the

Table 1 Lateral resolution of the magnetic projection photoelectron microscope for various electron energies and central magnetic fields

Electron energy (eV)	Expected lateral resolution (μm) for central magnetic fields (T)				de Broglie wavelength (μm)
	1	4	8	16	
500	8.0	2.0	1.0	0.5	5×10^{-5}
5	0.8	0.2	0.1	0.05	5×10^{-4}
0.05	0.08	0.02	0.01	5×10^{-3}	5×10^{-3}

photoelectron²⁵. The information depth is between 1 and 20 layers. The depth resolution in photoelectron microscopy is thus comparable with Auger electron microscopy and much higher than scanning electron microscopy. This high depth resolution means that in the study of metal surfaces for example, only the top few layers of atoms will contribute to the image. For biological surfaces, a depth of information of a few nanometres would permit a plasma membrane to be imaged while discriminating against the cytoplasm (see, for example, ref. 26). Depth of information is a function of incident light wavelength, angle of incidence and of any surface monolayers. Use of monolayer coating with a judicious choice of incident light parameters might further reduce the depth of information.

Energy resolution

By observing changes in the image as a function of the secondary electron energy distribution, scanning electron microscopy can, for example, discriminate between features whose secondary electron yield functions differ by as little as 0.5 eV. Con-

ventional UV photoelectron spectroscopy, however, has afforded energy discrimination as small as a few meV when isolated molecules are photoionized in the vapour phase²⁷ and, although band broadening effects in solids generally make the extremely high resolution of such experiments unnecessary, the possibilities for mapping a surface with such high energy resolution are extremely attractive. For example, sharp peaks (< 0.1 eV) have been seen in angle-resolved photoelectron spectra from surface states in copper²⁸ and gold²⁹. Some semiconductors and surface layers of weakly adsorbed species also exhibit narrow bands. Using the present instrument as a retarding potential analyser for gases gives an energy resolution of 20 meV—this may be capable of further improvement.

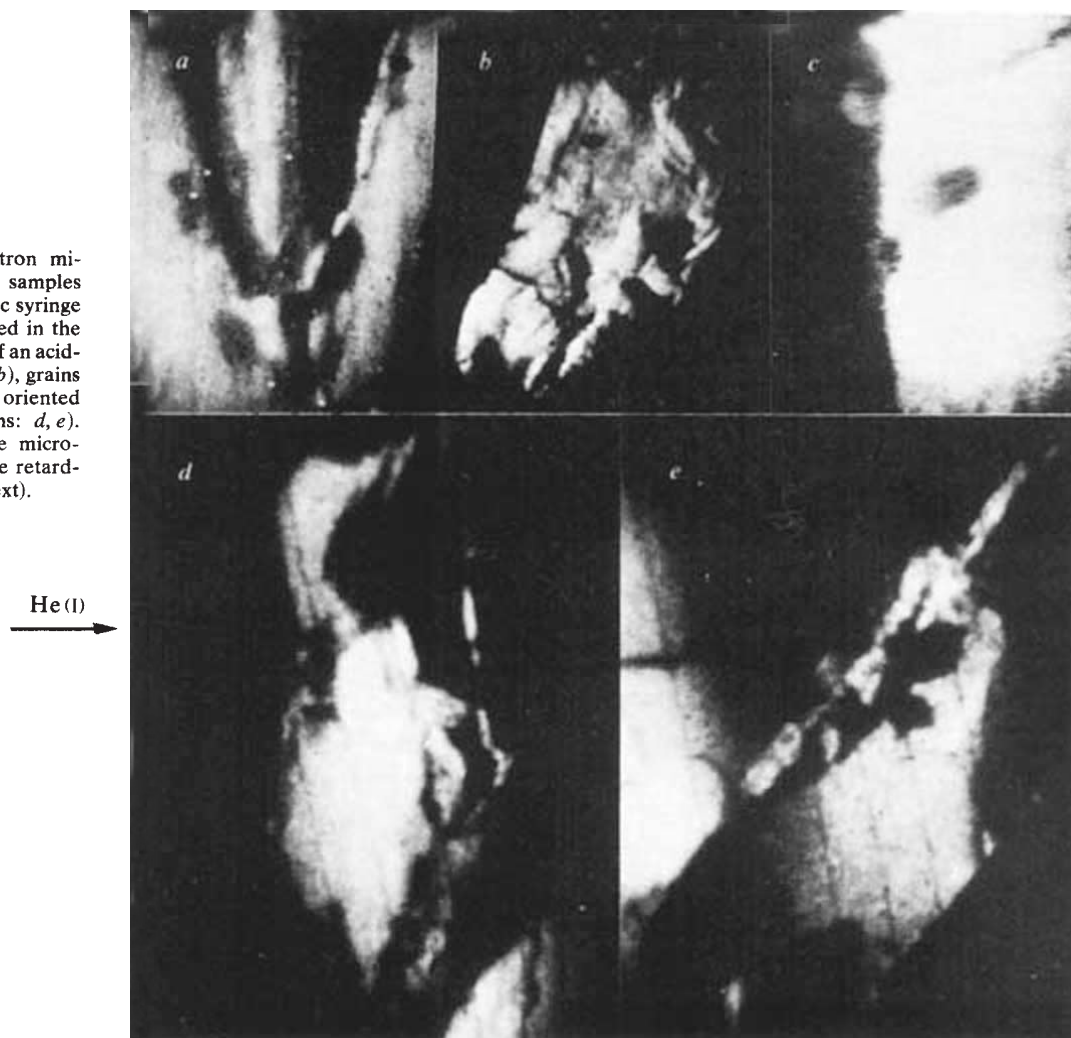
The present instrument

Figure 2 illustrates the present instrument. The divergent magnetic field is produced by a 'split-pair' superconducting solenoid whose central field can vary from 0 to 7.7 T. A gold-coated cubic electrode structure at the centre of the solenoid establishes a unipotential region, with various apertures for a 1-mm diameter collimated photon beam (initially of 21.2 eV photons from a He(I) d.c. discharge lamp), a sample probe, and for the emergent photoelectrons. The probe bearing the solid sample is mounted on the arm of a vacuum manipulator and can be translated and rotated.

Outside the solenoid, the magnetic field strength along the instrument axis is controlled using a magnetic shielding cage within the electron flight tube, and a system of axial and transverse correction coils outside the flight tube.

The magnetic shielding cage is an assembly of 24 gold-coated silver steel rods which reduce the magnetic field produced by the

Fig. 3 He(I) photoelectron micrographs of some test samples mounted on a hypodermic syringe needle. Brass wire inserted in the small hole (a). The edge of an acid-etched copper fragment (b), grains of pollen (c), a flake of oriented graphite (two orientations: d, e). Dark lines seen in these micrographs are shadows of the retarding grid wires (see text).



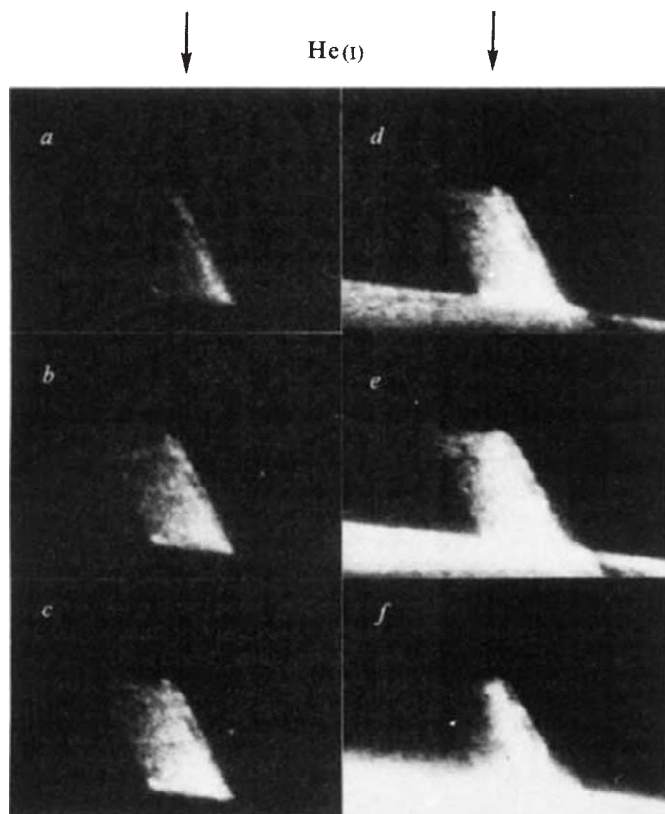


Fig. 4 He(I) photoelectron micrographs of a fragment of crystal-line chromium trisacetyl acetate attached to a stainless steel needle using electrons of energy greater than: *a*, 14 eV; *b*, 13 eV; *c*, 12 eV; *d*, 11 eV; *e*, 10 eV; *f*, 0 eV. Magnetic field strength 5 T. Thickness of the crystal $\sim 100 \mu\text{m}$.

superconducting solenoid within the cylinder. This, together with the external axial coils, is designed to establish a field which falls from a value of the order of 0.01 T at the nearest end to ~ 0.001 T at the furthest when the main field is at its maximum value. This large field reduction provides a magnification of the order of 100 and electron energy resolution is limited only by surface micropotentials (see Fig. 2 of ref. 1). With the present

arrangement, the argon photoelectron spectrum obtained in integral mode shows an energy resolution of ~ 20 meV. The resolving power of the retarding analyser is approximately constant in the 1–10 eV kinetic energy range.

A secondary function of the gold-plated silver steel rods is to establish a uniform transverse electrostatic field as an alternative way of energy analysis. Mounted at the front end of the shielding cage is a gold-coated tungsten wire grid with 95% transparency which, together with a system of gold-coated stainless steel rings, establishes a planar retarding field energy analyser. At the outer end of the shielding cage is the electron detection and imaging system. This consists of a 2-inch diameter microchannel plate (25- μm channel spacing) and low energy phosphor screen.

The lateral resolution of object detail is limited to $\sim 2 \mu\text{m}$ mainly by the coarseness of the 'home-made' phosphor screen and vibrations at the object. Ultimately, the limit will be imposed by the channel plate structure and the finest channel plate structure available (12 μm) would correspond to a lateral resolution of the object of 0.12 μm when $B(1)/B(2)$ is 10^4 . The photographs in Figs 3–7 are degraded by marked 'blooming' in the TV image close to features having high intensity. Both of these defects should be removed by using a charge-coupled electron image sensor.

The He(I) photoelectron count rate at the phosphor screen is typically 50,000 c.p.s. from argon at 2×10^{-5} torr. With a solid sample, the electron flux is too large for electron counting. The image on the phosphor screen is observed by a television camera and can be photographed directly from a video monitor. Alternatively, the camera output can be digitized and stored in a micro-computer, either as the whole picture frame or just one line or section of a line. In the latter case, the computer is used to advance the retarding potential on the analyser as successive frames are scanned and so to build up an array of spectra along a particular picture line segment. This array of integral spectra can be processed to yield the more familiar form of differential photoelectron spectra (see, for example, Fig. 7).

The vacuum chamber of the instrument is pumped by a 5-inch diffusion pump through a water-cooled baffle and liquid nitrogen trap. Thus a background pressure of 10^{-7} torr is readily achieved when the helium lamp is operating.

Although the present instrument has no vacuum lock for sample changing nor a separate argon ion gun for surface etching, *in-situ* cleaning is straightforward, with the strong magnetic field playing an important part. When argon is admitted to the working region to a total pressure of $\sim 100 \mu\text{m}$, a

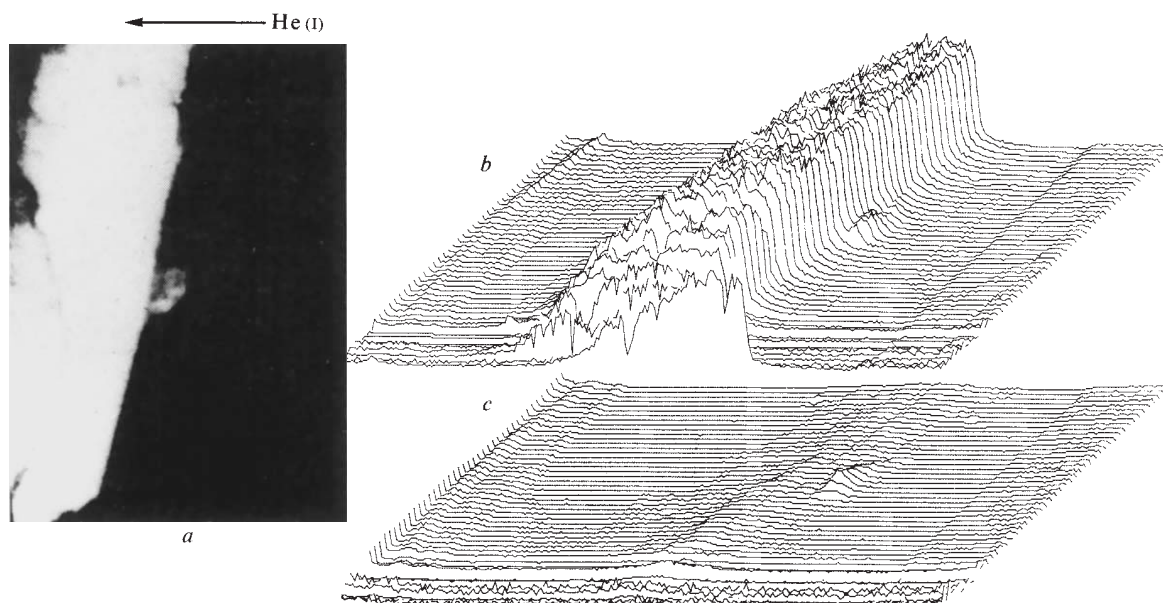


Fig. 5 *a*, Helium (I) photoelectron micrograph of pollen grains attached to a syringe needle. *b*, *c*, Digitally accumulated pictures of the same subject using electrons of energy greater than 5 eV (*b*) and 13.5 eV (*c*).

Penning-type glow discharge is established with ~ 400 V between the sample support and the surrounding cage. A current of ~ 0.1 mA for a few minutes causes rapid changes in the images of both metallic and plant material surfaces.

Examples

As a preliminary demonstration, a few fragments of mineral and biological origin were attached to the top of a hypodermic syringe needle placed on the manipulator. Figures 3–7 show images obtained by photographing the normal TV image on a monitor screen together with their electron energy distributions along a chosen TV raster line. The direction of illumination by the He(I) beam is indicated.

Figure 3 shows a haphazard collection of objects imaged using electrons of all energies, that is the retarding potential of the analyser was set to zero. The images are (a–e) of a 250 μm brass wire, the edge of an acid-etched annealed copper foil fragment, some pollen grains and two views of a flake of 'oriented graphite' (UCAR) stuck tangentially to the needle surface.

Images of a microcrystal of chromium (III) triacetylacetate ($\text{Cr}(\text{Acac})_3$) some 100 μm thick obtained using energy analysed electrons are shown in Fig. 4. The image of the supporting surface (stainless steel) could be completely suppressed by observing only high energy electrons (> 11 eV). Much more significant is the variation of relative brightness of the different crystal faces at different voltages. One face in particular (the right-hand edge in Fig. 4), which is both illuminated and observed at near grazing incidence, remains visible in photoelectrons of energy 17 eV. At low electron energy the end of the crystal facing the beam is the strongest emitter (this is not clearly shown in the present series of photographs because the vidicon was overloaded).

Fragments of plant material (pollen grains, cellular fragments and fibres) showed similar behaviour when observed immediately after sample insertion and initial evacuation, or after brief argon ion bombardment. But the maximum kinetic energy for photoelectrons was generally lower (~ 14 eV) although higher energy photoelectrons could be observed from selected areas with carefully controlled ion-cleaning. Figure 5a shows the > 10 eV photoelectron image of a pair of pollen grains (diameter ~ 50 μm) on the stainless steel surface together with computer-stored images obtained using > 5 eV (Fig. 5b) and > 13.5 eV (Fig. 5c), photoelectrons. The pollen grains (of *Echinopsis eyriesii*) have slightly dimpled spherical surfaces but the images show a marked spatial dependence of the energy distribution.

In a more complicated plant fragment (of poppy anther), an interesting variation of electron energy distribution along a selected line can be discerned (Fig. 6d) related to the intensity distribution in the different images obtained as the retarding potential varies (Fig. 6a, b, c).

Figure 7 shows the change in photoelectron emission from an acid-etched copper surface as it is cleaned by ion bombardment. Figure 7a is an all-photoelectron micrograph of the copper surface before cleaning. Figure 7b–d shows the changes which occur in the electron energy distribution along the arrowed line in Fig. 7a as the surface becomes cleaner. Figure 7b shows the uncleaned surface. Figure 7c, d shows the progressive changes after Ar^+ bombardment. A low-electron energy band is progressively lost. The final observed spectra do not have the appearance of conventional photoelectron spectra of copper surfaces for several reasons. First, although the annealing and acid etching did afford a surface topography showing the presence of different crystallites, the exposed faces were ill-defined crystallographically. Second, the distribution is not angle resolved. In effect we have integrated the spectra over the emitting hemisphere. This is, of course, a feature of the magnetic projection. All emitted photoelectrons are collected. Third, in this preliminary study the surface is not completely clean even after ion bombardment, in particular the Fermi edge is still suppressed.

The bright line on the left of Fig. 7a is photoemission from the epoxy resin used to mount the copper sample, together with its shadow. The difference in contrast arising from the different photoelectron energy distribution is quite striking at certain analyser voltages.

Conclusions

We have demonstrated that photoelectron images of microscopic surface structure can be obtained using energy-analysed electrons. The He(I) resonance line was used for the demonstration because it is easy to generate and has been widely used in the study of surface valence and photoemission. For element mapping, however, other resonance lines could be used. For core electrons, in particular, soft X-ray lines and synchrotron radiation may have advantages where it is possible to match the photon energy to a suitable shell or sub-shell ionization energy of the required element. This technique thus has the unique ability of producing either a molecular or atomic map of the surface depending on the photon energy. Scanning Auger

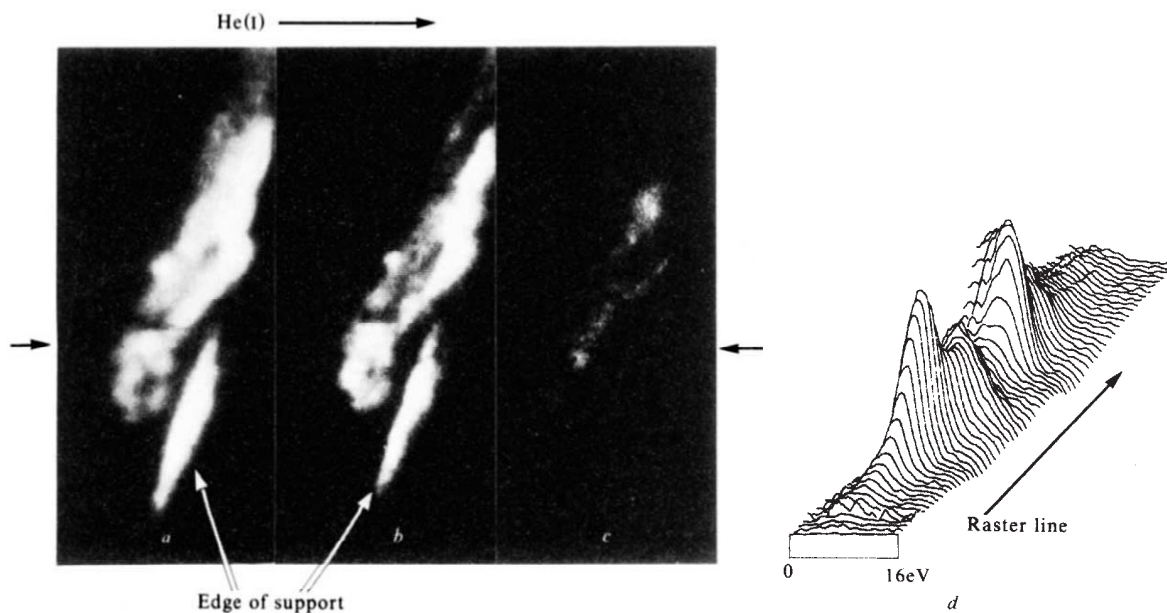


Fig. 6 a, b, c, He(I) photoelectron micrographs of a fragment of poppy anther using: a, all electrons; b, electrons of energy > 8 eV and c, electrons of energy > 11 eV. Magnetic field strength, 4 T. d, Photoelectron energy spectra (16 eV energy scale) along the raster line marked by arrows.

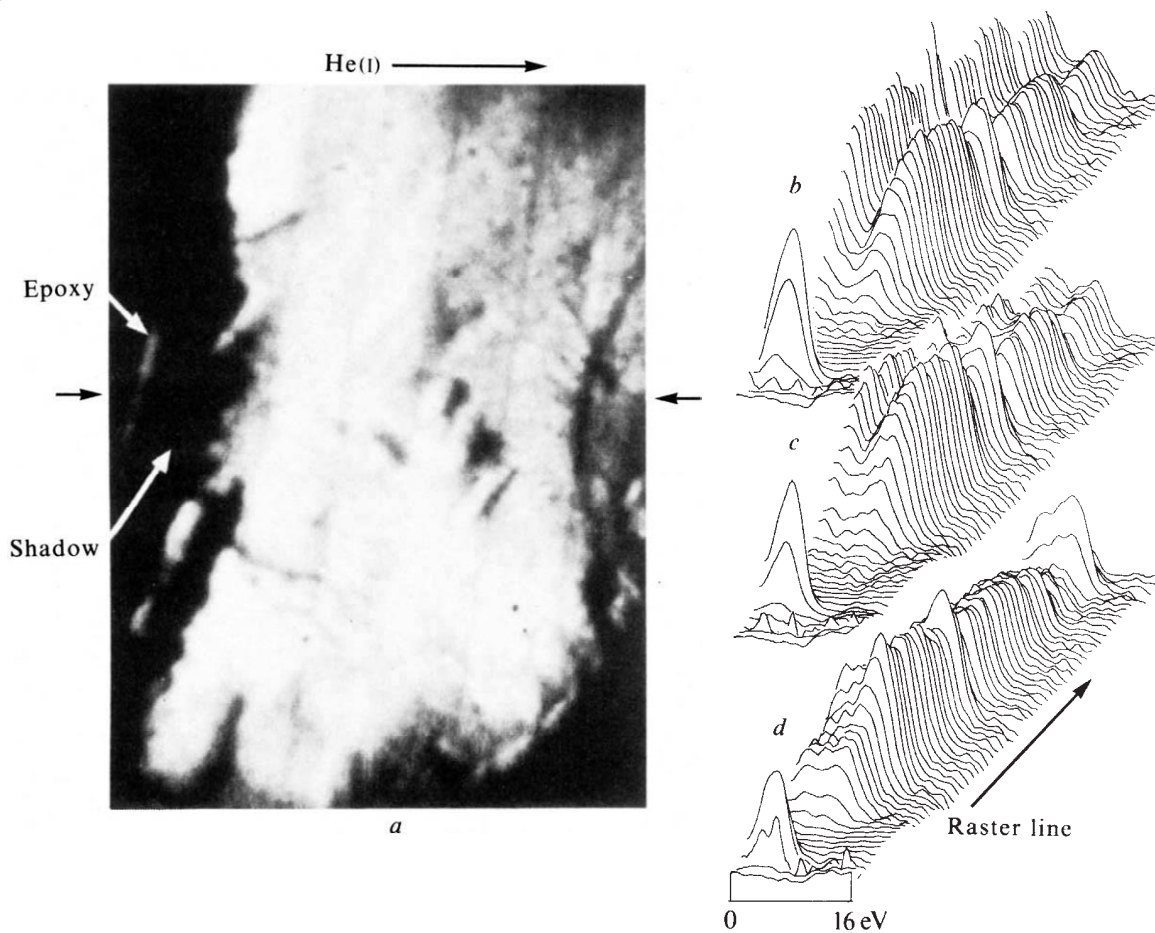


Fig. 7 *a*, He(I) photoelectron micrograph of an acid-etched copper surface before ion bombardment. Magnetic field strength, 7 T. *b*, *c*, *d*, The changes in photoelectron energy spectra along the raster line marked by arrows, as the surface is progressively cleaned by argon ion bombardment.

microscopy, on the other hand, provides only an atomic or elemental map of the surface. By using a photon energy only slightly in excess of the ionization energy both lateral resolution and sensitivity will be at the optimum. When the sample is irradiated near grazing incidence, photoemission is concentrated in the first atom layer and depth resolution is then at its optimum.

A most important advantage may, however, be derived from the much less destructive nature of the photoemission process combined with the high electron collecting efficiency.

To obtain good lateral resolution in Auger microscopy a current density of 10 A cm^{-2} is required²⁴. In such irradiation electron trapping, charging and secondary electron emission are important and the following electron-induced artefacts can be observed: migration of surface ions, decomposition, electron-induced desorption and diffusion induced by local Joule heating. The power dissipated in the sample by the He(I) discharge lamp is estimated to be less than that in the Auger electron micro-

scope by a factor of 1,000 or more. The advantage may be considerably larger, when the reduction in chemical damage associated with photons compared with electrons is taken into account.

The use of a very strong magnetic field, though primarily instrumental in intent, also has important possibilities for the study of energy bands and spin polarization in magnetic materials. This, when combined with the imaging property, may be particularly valuable in materials science in the study of magnetic domains. Photoemission from a ferromagnetic material in which the domains have been aligned by an external 0.05 T field is known to lead to spin-polarized photoelectrons³⁰.

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Identification and nucleotide sequence of a diversity DNA segment (D) of immunoglobulin heavy-chain genes

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A putative diversity segment of immunoglobulin heavy-chain genes (D segment) has been identified 700 base pairs 5' to J_H DNA on the germ-line genome of the mouse. This 10-base pair D segment is flanked by two sets of sequences related to

CACTGTG GGTTTTGT
GTGACAC CCAAAAACA

which are possible recognition sites for a recombinase. The spacer separating the heptamer and the nonamer is 12 base pairs long on both sides of the D segment. As the spacer separating the two signal sequences in V_H DNAs and J_H DNAs is 23 ± 1 base pairs long, the two recombinations required for creation of a complete immunoglobulin V_H gene, a V_H-D joining and a D-J_H joining, follow a 12/23-base pair spacer rule. Allelic exclusion is discussed with respect to D segments.

It is well established that complete immunoglobulin variable region genes (V genes) are generated by somatic DNA recombination(s) during the development of B-lymphocyte cells¹⁻¹⁵. In the light-chain genes of the mouse, in both λ- and κ-types, the last 13 COOH-terminal residues of the variable region are encoded by a separate DNA segment called J DNA (J for joining) in the germ-line genome^{4-7,9,10}. Coding information for the rest of the V region is contained in an embryonic V DNA segment^{3,6,7,9,10}. Accordingly, somatic recombination occurs between the 3' end of the V DNA and the 5' end of the J DNA to generate a complete light-chain V gene^{4,6,8}. The Jλ₁ (ref. 6) and five Jκ DNAs^{9,10} carry two blocks of sequences, one a palindromic heptamer, CACTGTG, and the other a nonamer, GGTTTTGT, highly conserved in the 5'-noncoding regions. These conserved sequences are invertedly repeated in the 3'-noncoding regions of all embryonic Vλ and Vκ DNAs sequenced to date^{3,6,7,9,10}. Another striking feature around the recombination site is that the spacer between the heptamer and the nonamer^{12,13} in all V and J DNAs is 12 ± 1 or 23 ± 1 base pairs long. In addition, recombination seems to occur only between a V DNA with the shorter (12-base pair) spacer and a J DNA with the longer (23-base pair) spacer or vice versa (12/23-base pair spacer rule). Based on these observations

Early *et al.*¹² and Sakano *et al.*¹³ predicted that the same or a similar enzyme mediates the recombinations in both λ- and κ-light-chain genes and that the recombinase consists of two functionally distinct units, one recognizing the heptamer and the nonamer separated by the shorter (12-base pair) spacer and the other recognizing the same signal sequences separated by the longer (23-base pair) spacer.

In contrast to the light-chain genes, heavy-chain J DNAs (J_H) do not seem to associate directly with the heavy-chain V DNAs (V_H). In the case of the γ2b heavy-chain gene expressed in myeloma MOPC141, the 14-residue peptide comprising the third hypervariable region (HV3) beginning with Val (residue 98) and ending with Thr (residue 111) is encoded neither in the germ-line V DNA nor in the J DNA¹³. A similar observation has been made independently in an α heavy-chain gene¹². Therefore, it is generally believed that the HV3 region of a heavy chain is encoded by a separate DNA segment, D (D segment for diversity¹⁶), and that two recombination events, V_H-D and D-J_H joinings, are necessary to form a complete heavy-chain V gene. All germ-line V_H DNAs and J_H DNAs sequenced so far have the conserved heptamer and nonamer separated by the longer spacer. It has therefore been predicted^{12,13} that germ-line D segments must contain the same recognition sequences on each side of the coding segment and their spacers must both be short ones, so that the 12/23-base pair spacer rule is adhered to. However, no germ-line D sequence has been reported to date and the predictions outlined above still need to be verified. It was previously observed that the DNA sequences are often rearranged in the J_H cluster in some cloned T-cell lines^{17,18}, as well as in pre-B-cell lines^{18,19}. By the Southern hybridization method using a J_H cluster probe, we observed that DNA rearrangements within the J_H cluster region are not restricted to one allelic chromosome in most of the myelomas. One intriguing possibility was that some of these rearrangements were abortive, representing recombination between a D segment and a J_H segment producing an incompletely rearranged D-J_H structure. We therefore cloned some of the genomic DNA fragments containing the rearranged J_H-cluster sequence from myelomas and characterized the cloned DNA fragments. These studies led to an identification of a potential germ-line D DNA segment, which is reported here. This potential D DNA segment is flanked by the two pairs of conserved sequences in inverted orientations, and the spacers separating the two signal sequences are 12 base pairs long for both.

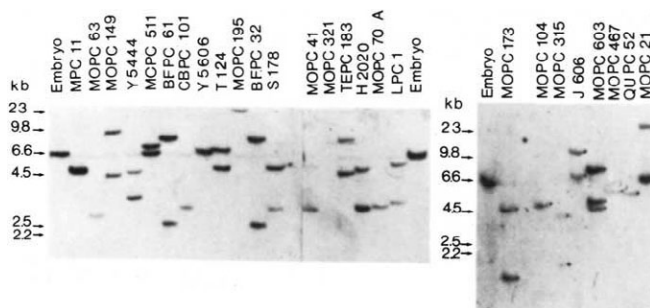
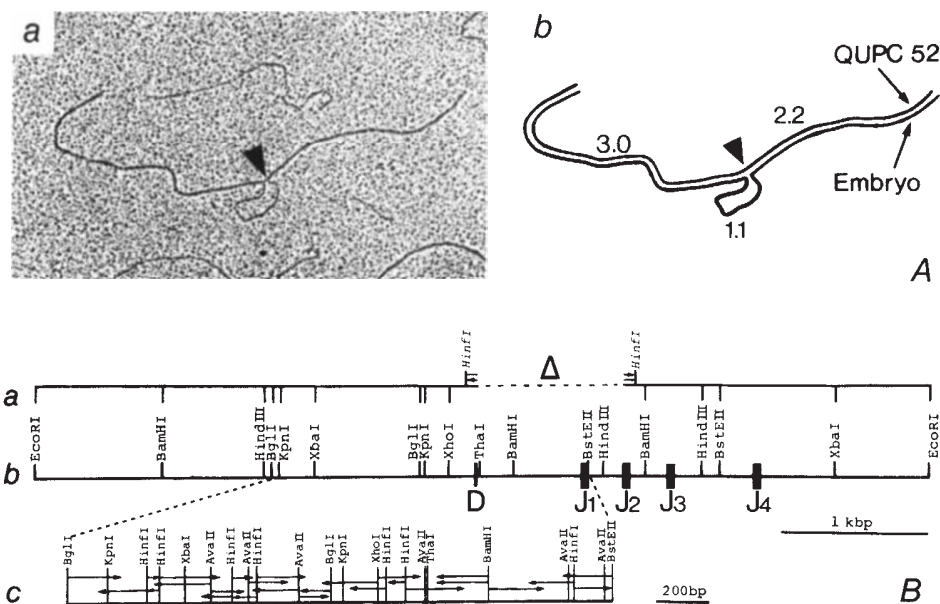


Fig. 1 Southern blot analysis of mouse embryo and myeloma DNAs. Mouse DNAs were digested with *Eco*RI, separated in 0.8% agarose gels and blotted to nitrocellulose filters according to the method of Southern²⁰. Filters were then incubated with the nick-translated J_H probe (1.1-kilobase *Sac*I-*Eco*RI fragment, see ref. 11), essentially as described by Wahl *et al.*³⁹. *Hind*III fragments of phage λ DNA were used as size markers (in kilobases, kb). For the cloning of a 5.2-kilobase Q52 fragment hybridizable with the J_H probe, *Eco*RI digest of the myeloma DNA was fractionated in a 0.8% agarose gel and DNA from the J_H probe-positive fraction was eluted as described previously⁴⁰. Recombinant phages containing the mouse DNA were screened by *in situ* plaque hybridization⁴¹ with the J_H probe.

Fig. 2 A, Electron micrograph (a) and schematic interpretation (b) of a heteroduplex molecule formed between two *Eco*RI inserts, one from clone ME184-8 (embryonic J) and the other from clone Q52J (rearranged J from myeloma QUPC52). Procedures used for the heteroduplex formation have been described previously⁴. Clone ME184-8 was prepared by Richard Maki and contains, in a phage λ_{WES} vector, the 6.4-kilobase *Eco*RI embryo DNA fragment carrying the four J_H DNA segments whose sequences were determined previously¹³ using another DNA clone referred to as MEP203 (ref. 11). Numbers indicate lengths of various parts of the heteroduplex in kilobases. B, Restriction enzyme cleavage maps of the J-containing *Eco*RI fragments. a, 5.2-kilobase insert from the myeloma clone Q52J; b, 6.4-kilobase insert from the embryonic clone ME184-8. The broken line (Δ) in Ba represents a deletion in the myeloma clone; the bars in Bb indicate coding regions. The positions of four J_H segments have been determined by R-loop mapping and DNA sequencing^{11,13}. The D segment is identified in the present study by DNA sequencing of the 5' flanking region of J_{H1} . Strategy of the sequencing is shown in Bc. kbp, Kilobase pairs; bp, base pairs. The previously published *Bam*HI sites¹³ have been corrected.



Frequent rearrangements of the J_H region sequences in myelomas

We analysed *Eco*RI digests of total DNA from 18 different BALB/c myelomas by the Southern gel blotting method²⁰ using a J_H probe (Fig. 1). The embryo DNA gave a band of 6.4 kilobases as expected from previous studies¹¹. All but three (MOPC511, MOPC21 and MOPC321) myelomas gave one, two or three bands that are all different from the embryo band. MOPC511 and MOPC21 each gave two bands, one of which is indistinguishable from the embryo band. MOPC321 gave no band. This myeloma synthesizes no heavy chain and presumably lacks part of the heavy-chain gene sequences in question. In some myelomas a very faint band can be seen at the position of the embryo band, probably due to contamination of non-myeloma cells in the solid tumours from which the DNA was prepared. The variability in the number of bands among various myelomas and in the intensity of bands within a myeloma most probably reflects the fact that myelomas are usually not diploid²¹.

We assume that one rearranged band in each myeloma represents the V_H -D- J_H joining required for the formation of a complete heavy-chain gene active in that myeloma. Although the nature of the additional rearrangements observed in many myelomas is unknown, the results show that sequence rearrangements are very frequent in the vicinity of the J_H cluster in all copies of chromosome 12, on which the heavy-chain genes reside²².

DNA deletion on an abortively-rearranged J_H fragment in myeloma QUPC52

We chose the 5.2-kilobase QUPC52 (Q52) fragment for further analysis and cloned it in the phage vector λ_{WES} (ref. 23). The cloned *Eco*RI fragment was analysed by heteroduplex formation using a germ-line J_H fragment (clone ME184-8) cloned from the *Eco*RI digest of mouse embryo DNA. As shown in Fig. 2A, the 5.2-kilobase myeloma fragment carries a 1.1-kilobase deletion, which lies 3.0–4.1 kilobases from the 5' end of the embryonic J_H fragment. By superimposing the heteroduplex map on the restriction enzyme map of the embryonic clone, we could locate the right end of the deletion near the J_{H2} DNA and the left end about 0.7 kilobases 5' to the J_{H1} DNA (Fig. 2B).

D-like segment is attached to J_H DNA on the abortive clone

To analyse the structure of the DNA segment joined to the J_H region, we purified a 600-base pair *Bgl*II-*Bam*HI fragment (see Fig. 2Ba) from the myeloma clone and determined the nucleotide sequence. Figure 3b shows the nucleotide sequence of a 110-base pair *Hinf*I-*Hinf*I fragment containing the deletion. As expected, we found the J_{H2} sequence in the sequenced region. By comparing this myeloma sequence with the germ-line J_{H2} sequence (Fig. 3b, c) we found that the J_{H2} sequence starts with the third nucleotide of the Asp codon at position 101 (number-

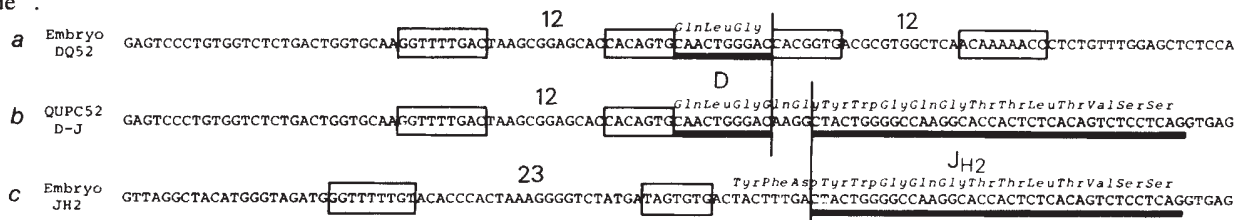


Fig. 3 Nucleotide sequence of the D-J structure in a myeloma clone, Q52J (b), and its germ-line sequences, J_{H2} (c) and D_{Q52} (a). For the sequencing of b, a 110-base pair *Hinf*I-*Hinf*I fragment containing a deletion was purified from a 600-base pair *Bgl*II-*Bam*HI fragment (see Fig. 1Ba), both ends labelled with [α -³²P]-nucleoside triphosphates, and the strand separated and sequenced in both directions according to the procedures described previously⁴². The embryonic J_{H2} sequence (c) was taken from ref. 13. The embryonic D_{Q52} region (a) was sequenced according to the strategy shown in Fig. 2Bc. Two blocks of conserved sequences,

GGTTTTTGT and CACTGTG
CCAAAAACA and GTGACAC

or their related sequences are boxed. Numbers indicate lengths of spacers separating the two conserved sequences in base pairs. Sequences in a and c contributing the coding region in b are underlined. The corresponding sequences in b are also underlined. Note that tetranucleotide AAGG in b between the two boundaries of D_{Q52} and J_{H2} is not accounted for by either germ-line sequence. Vertical lines indicate possible recombination sites with D_{Q52} and J_{H2} . Encoded amino acid sequences are in italics.

Fig. 4 Nucleotide sequence of the 5'-flanking region of J_{H1} DNA. Strategy for the sequencing is shown in Fig. 2Bc. The J_{H1} sequence and some of the 5'-flanking sequence have been published elsewhere^{12,13,43}. The D_{OS} and J_{H1} segments are underlined. Two blocks of conserved sequences,

or their related sequences are boxed. Numbers indicate the lengths of the spacer separating the two conserved sequences in nucleotide bases.

If the rearrangement on the Q52 clone represents the deletion between a D segment and a J_H segment, the germ-line D sequence must be on the 6.4-kilobase embryonic J_H fragment. As shown in Fig. 2, the left end of the deletion on the Q52 clone has been mapped 0.7 kilobases 5' to the J_{H1} DNA on the germ-line clone. We therefore extensively sequenced the 5'-flanking region of the J_H DNA cluster on the embryonic clone. Figure 4 shows the complete nucleotide sequence of the 1-kilobase region upstream from J_{H1} DNA. As predicted, about 700 base pairs 5' to the J_{H1} we identified the same sequence as found on the 5' side of Q52 J_{H2} DNA. In this germ-line sequence another set of the palindromic heptamer and the T-rich nonamer are invertedly repeated on the 3' side (Figs 4 and 3a). Again the spacer separating the heptamer and the nonamer is 12 base pairs. Accordingly, this germ-line sequence has the structure predicted for the germ-line D segment. In the D-like segment identified here, a 10-base pair DNA stretch is flanked by two palindromic heptamers.

and further flanked by A + T-rich nonamers.

12 base pairs outside. This 10-base pair segment can code for three amino acid residues, Gln-Leu-Gly, Asn-Trp-Asp or Thr-Gly-Thr. Although no published mouse HV3 sequence cor-

The tetranucleotides, AAGG, adjacent to the recombination site on the Q52 J_{H2} DNA cannot be explained by either the germ-line J_{H2} or the D_{O52} sequence (Fig. 3), and at present the origin of this sequence is unknown. We looked for another germ-line D segment encoding the tetramer between the D_{O52} and J_{H1} sequences and found none (Fig. 4). As shown in Fig. 3a, the coding sequence in the embryonic D_{O52} segment is followed by pentanucleotides CACGG. If we assume one nucleotide deletion and one nucleotide substitution in this pentamer, the tetramer AAGG could be generated. These events might have occurred somatically on recombination (see the accompanying article¹⁷ for more discussion on this point).

In the hope of finding another D segment, we extensively sequenced the 1.3-kilobase segment 5' to D_{Q52} according to the strategy shown in Fig. 2Bc (sequence not shown). However, detection of another D or D-like sequence was unsuccessful: therefore, germ-line D segments do not seem to be as tightly clustered as are J_H DNAs.

The present study extended the 12/23-base pair spacer rule^{12,13} to germ-line D segments. It thus seems that all recombinations leading to formation of a complete V gene are mediated by the same or similar enzymes. These enzymes are thought to contain two DNA-binding proteins, one for a DNA segment carrying the heptamer and nonamer separated by a short (12-base pair) spacer and the other for a DNA segment having essentially the same short sequences separated by a long (23-base pair) spacer^{12,13}. As the two pairs of the putative recognition sequences are in an inverted orientation relative to the corresponding coding DNA segments, they could form a transient intermediary duplex if it is stabilized by the recombinase^{9,13}.

It seems to be a general rule that sequence rearrangement involving immunoglobulin genes accompanies a deletion of the DNA sequence which separates a pair of joining DNA segments on a germ-line genome. Experimental evidence for this rule was first obtained for V-J joining of the λ_1 -type light-chain gene⁹, and was confirmed in a subsequent experiment²⁵ in the κ -type gene. There is also evidence that deletions accompany heavy-chain joining^{18,26}. Furthermore, a number of groups reported results which strongly suggest that the same rule applies to the 'switch recombination' of heavy-chain genes^{27-30,44}. All the evidence is based on hybridization analysis of mouse DNA using

an appropriate probe. The heteroduplex molecule, formed between the embryonic J_H clone and the Q52 J_H clone, provides the most direct evidence for the involvement of deletion in D- J_H joining. As the size of the cloned DNA fragments corresponds well to those of the fragments detected by Southern gel blotting analysis, the deletion cannot be attributed to cloning artefacts (see Fig. 1 for clone Q52 J_H).

D- J_H joining and allelic exclusion

Unlike all previous autosomal gene studies, only the gene on one of the two homologous chromosomes is active in a given cell for immunoglobulin chains³¹⁻³³. This 'allelic exclusion' is manifested in several ways for light chains. A common form is that one copy of the chromosome contains a rearranged, complete gene, and the other retains the germ-line configuration⁴. Alternatively, both alleles are rearranged, but one abortively in several different forms (refs 5, 18, 33-36, 45-48 and P. Kennedy and S.T., unpublished). At the heavy-chain locus, rearrangements on both copies of the chromosome seem to be very common (Fig. 1), and lack of rearrangement as observed in light chains does not seem to be a common form of allelic exclusion. The demonstration that the heavy-chain locus includes D DNA segments suggests a unique way in which this locus can be abortively rearranged. As shown here for myeloma QUPC52,

the unexpressed chromosome contains a D-J segment. Although allelic exclusion at the heavy-chain locus could be manifested by other forms of abortive rearrangement observed in the light-chain loci, the existence of D segments may provide additional forms. One might imagine that in addition to D-J joining V-D joining can occur.

The analysis of myeloma DNA indicates that rearrangement in the vicinity of J DNA segments is much more frequent in heavy-chain genes than in light-chain genes. This distinction also applies to normal B cells: little, if any J_H was observed in the germ-line configuration³⁷, whereas unrearranged J_K is observed in significant amounts³⁸. The reason for this difference is not clear. However, it is reasonable to assume that the frequency of joining of two separate DNA segments increases as the number of segments increases and/or as the distance between them decreases. If there are many D segments (see the accompanying article¹⁷) and if they are close to J segments as D_{Q52} is, D-J joining could account for more frequent abortive rearrangement in the heavy-chain locus.

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Identification of D segments of immunoglobulin heavy-chain genes and their rearrangement in T lymphocytes

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The finding that the diversity (D) and joining (J_H) but not the variable (V_H) DNA segments of mouse immunoglobulin heavy-chain genes are joined in the DNA of some cloned cytolytic T cells, led to identification and sequencing of three different D DNA segments. Two segments identified on the embryo DNA carry on both the 5' and 3' sides two sets of characteristic sequences separated by a 12-base pair spacer, which have been implicated as recognition signals for a recombinase. The third segment, identified in a form joined with a J_H DNA segment in a T cell, carries the recognition signal on the 5' side. These results support the 12/23-base pair model for somatic generation of immunoglobulin V genes, and rule out the possibility that the cytolytic T cells use assembled V_H , D and J_H sequences to encode their antigen receptors.

THE variable region of an immunoglobulin chain is encoded in multiple DNA segments scattered along a chromosome of a

germ-line genome¹⁻¹⁰. These DNA segments are assembled into a continuous stretch with concomitant deletion of the spacer

sequences during differentiation of B lymphocytes. The somatic rearrangement of DNA sequences is considered to have key roles in both somatic amplification of antibody diversity and determination of the specificity of lymphocyte clones. In mouse light chains of both λ and κ types, two DNA segments, V and J, are separate in the germ-line genome and are joined in the lymphocytes which express these DNA segments¹⁻⁵. The V and J segments code for about 95 residues at the NH₂-terminal and about 13 residues at the CO₂H-terminal ends of the V region, respectively^{1,3-5}. In contrast, DNA sequencing analysis of heavy-chain genes suggests that the V_H region is encoded by three DNA segments referred to as V_H, D and J_H, and somatic joining of the three DNA segments is necessary to generate a complete heavy-chain V gene⁶⁻⁸. The D DNA segment encodes the third hypervariable region, which determines in part the shape and size of the antigen-combining site^{11,12}.

The abovementioned sequence rearrangement of immunoglobulin genes should occur during the development of B lymphocytes before the appearance of the first detectable immunoglobulins. Does an analogous or similar rearrangement occur in the precursors of T lymphocytes, which share a common stem cell with B lymphocytes? This question is particularly important because of the possible use of the immunoglobulin V gene pool by T cells for coding their antigen-specific receptors¹³⁻¹⁶. One approach to this question is to analyse DNA of cloned T cells by the Southern gel blotting procedure using a J DNA sequence as the hybridization probe. A V-J or V-D-J joining will be monitored by a difference in the size of DNA fragments detected in embryo (or kidney) and T-cell DNA. We have therefore applied this technique to several cloned cytolytic T cells, and show here that sequence rearrangements frequently occur in T cells in the vicinity of the J_H sequences. Similar findings have been reported by two other groups with T-lymphoma lines¹⁷ and cytolytic T-cell lines¹⁸. However, our further analysis of two T-cell clones by recombinant DNA techniques demonstrates that the rearrangements observed in these T-cell clones are D-J joinings rather than V-D-J joinings. This led to the identification of two D DNA segments on the germ-line genome. One of the three D DNA segments described here was found to be identical to the D segment that was independently identified in our laboratory during the parallel analysis of an abortively rearranged sequence observed in the myeloma QUPC-52 (ref. 19).

T-cell DNA sequence is frequently rearranged in the vicinity of the J_H cluster

We have previously identified a cluster of four J_H DNA segments⁷ on a 6.4-kilobase *Eco*RI fragment cloned from BALB/c embryos (clone MEP203)⁶. A 1.1-kilobase *Sac*I-*Eco*RI fragment derived from the 3' end (relative to orientation of transcription) of this fragment (a J_H probe) would detect, when used as a hybridization probe on Southern blots of cellular DNA digested with *Eco*RI, sequence rearrangements that have occurred within the 6.4 kilobase *Eco*RI fragment. The results of various cytolytic T-cell clones and three thymomas are shown in Fig. 1a. The cytolytic T-cell clones, except B6.1, were confirmed for their functional activity before extraction of DNA. The embryo or kidney DNAs of five different mouse strains—BALB/c, C3H, C57BL/6, AKR and DBA—all gave the expected fragment of 6.4 kilobases previously identified. The DNA of three cytolytic T-cell clones—DAS 1, D.FL.2, D.FL.13—and two thymomas—BW 5147 and T5—each gave a single band indistinguishable from that of embryos or kidneys, suggesting that no gross sequence rearrangement has occurred in these cells within the 6.4-kilobase *Eco*RI fragment. [The faint bands observed above the 6.4-kilobase band in D.FL.2 and D.FL.13 DNA are due to incomplete digestion as demonstrated by separate experiments (data not shown).] In contrast, DNA of six cytolytic T cells—C.SP.2, C.FL.1, B6.1, D.FL.1, D.FL.12 and D.FL.16—gave one additional band of 5.5, 5.3 or 4.0

Table 1 Germ-line and myeloma D sequences

<u>Germ-line D</u>	
D _{SP2.1}	TC <u>TACTATGGTAAC</u>
D _{SP2.2}	TC <u>TACTATGATTACG</u> AC
D _{Q52}	C <u>AAGTGGGAC</u>
<u>Myeloma D</u>	
S107	<u>TACTACGGTAGTA</u>
M603	<u>TACTACGGTAGTA</u> C
M167	G <u>GACTACGGTAATA</u> GCTACTTTG
MC101	AGG <u>GACTACGTTAGTA</u> GGTACGACCC
MPC11	GGGATT <u>TACTACAATAGTA</u> GCCC
M141	CGTCTCAATTAT <u>TACTACGGTCGTA</u> GCGACAAACTTCACTT
M173-II	TGCCCTCTAC <u>TACTACAGTTACA</u> <u>GCGGGGGTT</u>
H76	<u>CCGGGGGGTCCCC</u>

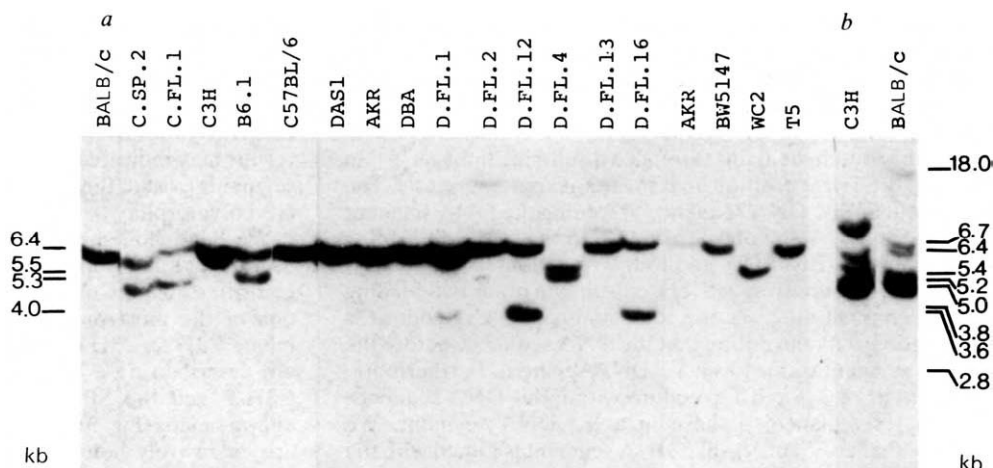
Three germ-line D sequences identified in the present study are compared with the D region sequences of the eight complete V_H genes present in the indicated myelomas. For D_{SP2.2} and D_{Q52}, the sequences between the palindromic heptamers are shown. The D_{SP2.1} has been identified only in a form joined with the J_{H3} segment and therefore its exact 3' boundary is unknown. For S107 (ref. 8), M603 (ref. 8), M167 (ref. 8), MC101 (ref. 27) and M141 (ref. 7) the DNA sequences of both the complete (rearranged) and the embryonic (unrearranged) V_H genes are known. These sequences and the embryonic J_H sequences⁷ were used to deduce the 5' and the 3' boundaries of these D regions. For MPC11 (ref. 28), M173-II (H.S. and Y.K., unpublished) and H76 (ref. 9), only the DNA sequences of the complete V_H genes are known and therefore the 5' boundaries are ambiguous. The 3' boundaries were deduced as above. All sequences are aligned for the maximal homology. The D regions of S107 and M603 contain a 13-mer (underlined) which is a perfect palindrome around the central G. The homology present between each of the other D sequences and the 13-mer is also indicated by underlining. The homology between H76 and M173-II D sequences is indicated by double underlining.

kilobases. This indicates that some of the two or more copies of chromosome 12 [some T cells are polyploid (H.v.B. and W.H., unpublished)] in these T cells have undergone sequence rearrangement involving the 6.4-kilobase embryonic fragment. In one cytolytic T-cell clone, D.FL. 4, and one thymoma, WC2, no 6.4-kilobase embryonic fragment was observed. The presence of two non-embryonic bands, of 5.5 and 5.3 kilobases, in D.FL.4 suggests that sequence rearrangement has occurred on both (assuming diploidy) chromosomal copies in different ways. In contrast, WC2 seems to contain only one copy of the 6.4-kilobase sequence and sequence rearrangement has occurred in this fragment. Thus, we have observed sequence rearrangement in 8 out of 13 cytolytic T-cell or thymoma clones. In the majority, rearrangement occurred only in some but not all of the copies of chromosome 12. The sizes of the rearranged fragments seem to fall into three categories—5.5, 5.3 and 4.0 kilobases.

Two DNA clones from two cytolytic T cells

We chose B6.1 and C.SP2 for further studies and cloned the rearranged DNA fragments of 5.5 and 5.3 kilobases, respectively, using a λ _{WES} vector²⁰; we refer to these fragments as B6B1 (short-term B6) and SP2B1 (short-term SP2), respectively. Electron microscopic examination of the heteroduplex molecules formed between either of these T-cell DNA clones and the 6.4-kilobase embryonic *Eco*RI fragment (clone ME183-8) allowed mapping of the recombination sites. The B6 and embryo DNA fragments gave a single-stranded DNA loop flanked by double-stranded DNA arms (Fig. 2a). In contrast, the SP2 and embryo DNA fragments gave Y-shaped heteroduplexes. Measurement of the various parts of the heteroduplex molecules indicated that the B6 DNA fragment has undergone a 1.2-kilobase deletion in a region immediately adjacent (5' side) to the J_{H2} sequence. In contrast, the entire sequence 5' to J_{H3} is

Fig. 1 *a*, Hybridization of T-cell and thymoma DNA with the J_H probe. High molecular weight DNA was prepared from various cloned cytolytic T cells and thymomas by the procedure described previously²⁹, digested to completion with *Eco*RI, and fractionated on 0.8% agarose gel by electrophoresis. The DNA was denatured and transferred *in situ* to nitrocellulose filters (Schleicher and Schuell, BA85) essentially as described previously³⁰. The filters were incubated for hybridization with nick-translated J_H probe (1.1-kilobase *Sac*I-*Eco*RI fragment indicated in Fig. 3A) according to the published procedures². Characteristics of the various cytolytic cells have been described previously^{31,32}. C.SP.2 and C.FL.1 are derived from C3H, B6.1 from C57BL/6, DAS 1 from AKR/DBA F1, all D.FL. clones from DBA, and thymomas were all derived from AKR. For comparison, DNA from BALB/c embryos and kidneys of C3H, C57BL/6, AKR and DBA were also analysed. These DNA are indicated by strain names. Numbers and arrows to the left indicate sizes of DNA fragments in kilobases (kb). *b*, Hybridization of BALB/c embryo and C3H kidney DNA with SP2D-J probe. A nitrocellulose filter, prepared as described for *a*, was incubated with the nick-translated SP2D-J probe, 0.8-kilobase *Bgl*II-*Hind*III fragment indicated in Fig. 3C. The sizes of the BALB/c DNA fragments detected are shown in kilobases. The 3.8-, 3.6- and 2.8-kilobase bands could be seen in the original autoradiogram, but are too faint to be reproduced.



replaced with a sequence of unknown origin in the SP2 DNA fragment. The points of sequence divergence between the T and embryo cell DNA fragments were further determined by comparison of restriction enzyme cleavage sites (Fig. 3A-C). The results confirm the conclusion drawn by heteroduplex mapping.

The SP2 fragment contains a joined D-J DNA segment

We determined the DNA sequence of the 0.8-kilobase *Bgl*II-*Hind*III fragment (Fig. 3Ca) of the SP2 fragment containing the site of recombination according to the strategy shown in Fig. 3Cb. This sequence was aligned with the previously determined sequence of the embryonic fragment in the J_H -coding region (Fig. 4). As suggested by the heteroduplex molecules (Fig. 2b) and the restriction enzyme maps (Fig. 3A, C), the sequences of the two DNA fragments match well starting with the first base of the Phe codon TTT located in the 5' edge of the J_H DNA segment. On the SP2 fragment the Phe codon is preceded by an Asn codon, AAC, which is in turn preceded by a nonamer, TACTATGGT, that codes for a trimeric peptide, Tyr-Tyr-Gly, when read in phase with the J_H . This nonameric nucleotide sequence or its close variants appear in the D regions of seven out of eight myeloma V_H genes sequenced to date (Table 1). Furthermore, the trimeric peptide, Tyr-Tyr-Gly, encoded by the nonamer has been found in the D regions of a majority of myeloma V regions for which amino acid sequence data are available¹¹. These data strongly suggest that a D DNA segment is joined with the J_H sequence on the SP2 fragment.

Further evidence for this notion comes from the feature of the nucleotide sequence located immediately 5' to the putative D DNA segment on the SP2 fragment. Based on the DNA sequencing analysis of the various germ-line DNA segments, it has been predicted that a nonamer,

GGTTTTTGT
CCAAAAACA

and a palindromic heptamer,

CACAGTG
GTGTCAC

separated by a 12-base pair spacer or closely related variants of these sequences, precedes a germ-line D DNA segment^{7,8}. As shown in Fig. 4, the putative D segment joined with the J_H on the SP2 fragment is preceded by two sequences,

GATTTTTGT and TACTGTG
CTAAAAACA ATGACAC

separated by a 12-base pair spacer. These results partially fulfill

the predictions of the 12/23-base pair spacer model^{7,8}, and in turn support the conclusion that the sequence joined with the J_H on the SP2 fragment is indeed a D DNA segment. We refer to this D DNA segment as $D_{SP2.1}$.

No V_H -D- J_H joining on the SP2 fragment

Is a germ-line V_H DNA segment also joined on the SP2 fragment? A visual inspection of the SP2 DNA sequence in the region preceding the $D_{SP2.1}$ segment indicates that no germ-line V_H or V_H -like sequence is attached to the D DNA segment. First, there are one, two or four translation termination codon(s) in the three different reading frames of the 300-base pair (an approximate size of a germ-line V_H segment) region 5' to the $D_{SP2.1}$ segment. Thus, this region cannot constitute part of a

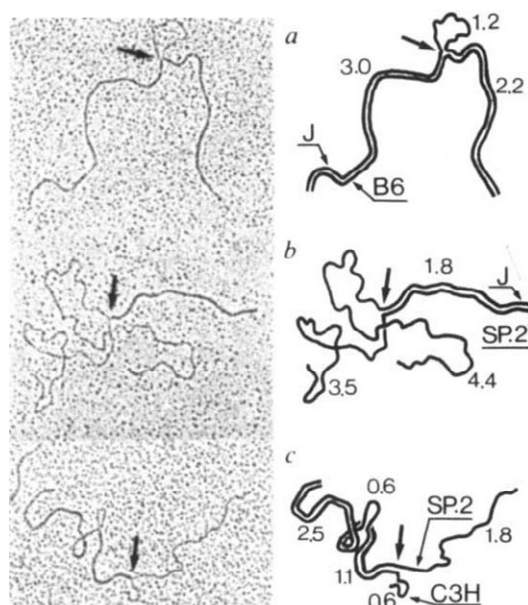


Fig. 2 Heteroduplex molecules formed between cloned DNA fragments. Cloned DNA was digested with *Eco*RI and examined under an electron microscope as described previously². Thick arrows indicate points at which heteroduplexes branch out. The numbers indicate lengths of various parts of the molecules in kilobases. In this experiment we used clone ME184-8 instead of MEP203^{6,7} as a source of the 6.4-kilobase embryonic *Eco*RI fragment. Clone ME184-8 was prepared by Richard Maki and contains, in a phage vector, λ_{WES} , the 6.4-kilobase *Eco*RI fragment carrying the four J_H DNA segments. *a*, 6.4-kilobase ME184-8 (J) versus 5.3-kilobase B6B1 (B6); *b*, 6.4-kilobase ME184-8 (J) versus 5.5-kilobase SP2B1 (SP2); *c*, 5.5-kilobase SP2B1 (SP2) versus 5.0-kilobase C3H-2 (C3H).

germ-line V_H DNA segment. Second, some amino acid residues are highly conserved among the V_H regions. In particular, Cys residues are invariably present in all species studied to date at positions 22 and 92 and are the only Cys residues in the V_H region¹⁰. These two Cys residues play an important part in folding the protein domains through a disulphide linkage^{21,22}. In addition, the Trp at position 36 is invariant across species¹¹. The 300-base pair SP2 DNA sequence preceding the $D_{SP2.1}$ segment contains three Cys and one Trp codons in the frame defined by the J_{H3} peptide. Eight Cys and four Trp codons, and four Cys and one Trp codons, respectively, exist in the other two reading frames. Thus, in all cases the total number of Cys codons is incompatible with the notion that the DNA sequence before the D DNA segment comprises a V_H DNA segment. Furthermore, the locations of Cys and Trp codons within this DNA sequence bear no resemblance to those in a V_H DNA segment. We conclude that no V_H or V_H -like DNA segment is joined with the D- J_H DNA on the SP2 fragment.

Identification of a $D_{SP2.1}$ -like D DNA segment in a germ-line genome

Identification of a D- J_H joining in the DNA of a cytolytic T-cell clone allowed the identification, cloning and characterization of germ-line D DNA segments. We dissected out the 0.8-kilobase *Bgl*II-*Hind*III fragment (Fig. 3C) from the SP2 clone and used it as a hybridization probe (SP2DJ probe) for analysis of *Eco*RI-digested total DNAs of both BALB/c and C3H embryos (Fig. 1b). As this probe contains the J_{H3} and its 3'-flanking sequence, it detects the 6.4-kilobase embryonic *Eco*RI fragment carrying

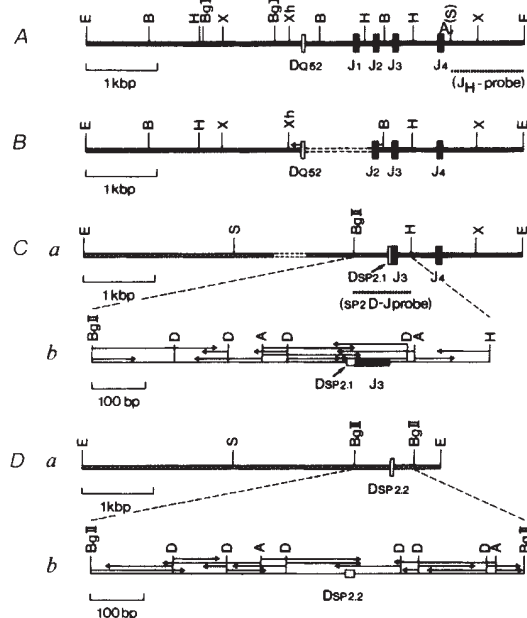


Fig. 3 Restriction enzyme cleavage maps of the *Eco*RI inserts of various DNA clones. **A**, The 6.4-kilobase *Eco*RI fragment of MEP203^{6,7}; **B**, B6B1; **C**, SP2B1; **D**, C3H-2. Clone MEP203, which was described previously^{6,7}, is an embryonic DNA clone and contains three *Eco*RI fragments, one of which is the 6.4-kilobase fragment carrying the four J_H segments. Cleavage sites were determined by single and double digestions of the cloned DNA by various restriction enzymes. Homologous regions are indicated by bars of two types of shading. The broken lines in **B** indicate the 0.6-kilobase region that appeared as a deletion loop in the heteroduplex molecules formed between the SP2B1 and C3H-2 fragments. The filled boxes correspond to the four J_H DNA segments previously identified on the MEP203 6.4-kilobase fragment^{6,7}. The open boxes are D segments identified by DNA sequencing. Sequencing strategy and finer restriction maps of the 0.8-kilobase *Bgl*II-*Hind*III fragment from SP2B1 and the 0.85-kilobase *Bgl*II-*Bgl*II fragment from C3H-2 are shown in **Cb** and **Db**, respectively. Horizontal arrows indicate the directions and extent of sequencing. The sequence across the 1.2-kilobase deletion of the B6B1 fragment was determined using the *Xba*I-*Bam*HI fragment (**B**). The J_H probe used in Fig. 1a and the SP2D probe used in Fig. 1b are indicated in **A** and **Ca**, respectively. Abbreviations used are: E, *Eco*RI; B, *Bam*HI; H, *Hind*III; Bgl, *Bgl*II; X, *Xba*I; Xh, *Xho*I; S, *Sac*I; BglI, *Bgl*II; D, *Dde*I; A, *Ava*II; kbp, kilobase pairs; bp, base pairs.

the four germ-line J_H segments. In addition, the probe detected one (5.0 kilobase), four (18, 6.7, 5.4 and 5.2 kilobase) and three (3.8, 3.6 and 2.8 kilobase) bands of high, medium and low intensity, respectively, in BALB/c DNA. A similar, but distinctive hybridization pattern was obtained in C3H DNA. These results may indicate that a number of $D_{SP2.1}$ or $D_{SP2.1}$ -like DNA segments exist in the germ-line genomes of mice, some of which are polymorphic.

We have cloned most of these DNA segments in phage λ vectors and determined their structure and organization in the germ-line genome (Y.K., unpublished). Some characteristics of one of the most prominent fragments, that of 5.0 kilobases (clone C3H-2, C3H in short-term) isolated from C3H embryos, are described here. The heteroduplex formed between clone C3H-2 and the SP2 fragment (Fig. 2c) and the restriction enzyme maps (Fig. 3C, D) indicate that the two DNA fragments are extensively homologous in the 3.6-kilobase region which starts with the 5' *Eco*RI end and ends somewhere near the position of $D_{SP2.1}$ on the SP2 fragment. However, that the homology is imperfect is clearly shown by the presence of a 0.6-kilobase insert (or deletion) in the C3H (or SP2) clone. Nevertheless, the above result suggests that the clone C3H may contain a $D_{SP2.1}$ -like DNA segment at the 3' edge of the homology. We therefore prepared a finer restriction map around this region and determined the entire DNA sequence of the 0.85-kilobase *Bgl*II-*Bgl*II fragment, according to the strategy shown in Fig. 3Db.

As predicted, the sequence homology between the SP2 and C3H fragments is extensive, starting with the 5'-*Bgl*II site and ending with the triplet AAC, the last codon of $D_{SP2.1}$ on the SP2 fragment (Fig. 4). There are 28 single-base substitutions or deletions (or insertions) in the 485-base pair homology region, two of which are within the D DNA segments. The D DNA segment on the embryonic clone C3H is flanked by two sets of the putative recognition sequences with 12-base pair spacers. This confirms the universality of the 12/23-base pair spacer model^{7,8}. Although the C3H-2 D DNA segment and $D_{SP2.1}$ are highly homologous, the imperfectness of the homology in the 5'-flanking regions as well as in the coding segments themselves clearly indicate that they are two different copies of the D segments that occupy independent positions in the germ-line genome. We therefore refer to the C3H-2 D DNA segment as $D_{SP2.2}$.

The B6 fragment contains another joined D-J sequence

Figure 5 shows the sequence across the 1.2-kilobase deletion of the B6 fragment and sequences of the embryonic fragment that cover the 5' and 3' boundaries of the deletion. The sequence homology between the B6 and the embryonic fragments ends with the C-G base pair immediately to the left of the line Ld and resumes with a T-A base pair immediately to the right of line Rd. The latter base pair corresponds to the second letter of the Phe codon TTT located near the 5' terminus of the previously identified J_{H2} sequence^{7,8}. The single base pair C-G present on the B6 fragment and flanked by the two lines, Ld and Rd, is unaccounted for by the embryonic sequence (see below). Ld is preceded by a putative enzyme recognition sequence,

GGTTTGTGAC (12-base pair spacer) CACAGTG
CCAAAAC TG GTGTCAC

both on the B6 and MEP203 fragments. On the latter fragment Ld is followed by nine base pairs, which are in turn followed by another putative signal sequence,

CACGGTG (12-base pair spacer) ACAAAAACC
GTGCCAC TGTTTTTGG

Thus, the 9-base pair sequence on the MEP203 fragment fulfills the requirement imposed on a D DNA segment by the 12/23-base pair spacer rule^{7,8}. We therefore conclude that the B6 deletion is due to the joining of the J_{H2} sequence with a D DNA



Fig. 4 Nucleotide sequences of three DNA clones: C3H-2, SP2B1 and MEP203 (refs 6, 7). All sequences were determined by the method of Maxam and Gilbert¹³. The sequence of the 0.85-kilobase *Bgl*II-*Bgl*II fragment of clone C3H-2 is aligned for the maximal homology with the sequence of the 0.8-kilobase *Bgl*II-*Hind*III fragment of clone SP2B1. Also aligned with the SP2B1 sequence is the sequence of the J_{H3} -containing region of clone MEP203, which was determined previously in our laboratory⁷. The SP2B1 fragment contains a D segment ($D_{SP2.1}$) fused with the J_{H3} segment. Amino acid residues encoded by the fused $D_{SP2.1}$ and J_{H3} on the SP2B1 fragment and those by the unfused J_{H3} on the MEP203, numbered according to Kabat *et al.*¹¹, are indicated in italics. The SP2B1 sequence is extensively homologous to the C3H-2 sequence from the 5' *Bgl*II site to the $D_{SP2.1}$ - J_{H2} boundary, where the homology switches to the MEP203 sequence. The dots and dashes indicate base substitutions and deletions within the homologous regions. The putative recognition sequences for recombinations are boxed. C3H-2 contains a D segment, $D_{SP2.2}$.

segment located 1.2 kilobases upstream. This D DNA segment has been independently identified during analysis of a rearranged fragment isolated from myeloma QUPC52 and is therefore referred to as D_{Q52} (see the accompanying article¹⁹).

As mentioned above, one base pair, C-G, is inserted on the B6 fragment at the site of recombination. Based on repeated sequencing of the relevant region, we believe that error is unlikely. This suggested the possible involvement of an intermediate recombination, the extra base pair arising from an independent D DNA segment. However, as described in the accompanying article, no additional D sequence has been identified between D_{Q52} and J_{H2} . Thus, if one is to pursue this possibility, it is necessary to invoke a scheme that allows joinings of multiple D DNA segments, the order of which in the assembled gene is independent of that in the germ-line genome. Although such a scheme is possible, one alternative is that the act of recombination introduced the extra base pair at the site of joining.

Structure and diversity of D sequences

In the digest of BALB/c mouse embryo DNA (Fig. 1b) the SP2D probe detected at least eight *Eco*RI fragments, each of which is likely to contain at least one copy of a $D_{SP2.1}$ -like DNA segment. For the 5.0-kilobase fragment, this was demonstrated directly by DNA sequencing, which led to the identification of $D_{SP2.2}$. Our recent result indicates that the 5.0-kilobase band contains three *Eco*RI fragments each of which hybridizes with the SP2DJ probe (Y.K., unpublished). Thus, assuming that positive hybridization with the probe signifies the presence of at least one D segment, the minimum number of $D_{SP2.1}$ -like segments is 10 (8 bands, of which one is triple).

If $D_{SP2.1}$ -like DNA segments are abundant, as the Southern gel blot suggests, this is in agreement with the frequent appearance of the $D_{SP2.1}$ -like sequences in the D regions of the

assembled V genes. The D-coding regions of seven out of eight assembled V_H genes sequenced to date contain a palindromic 13-mer, TACTACGGTAGTA (ref. 8), or its variants (Table 1). The $D_{SP2.1}$ and $D_{SP2.2}$ also contain sequences similar to the 13-mer (Fig. 4, Table 1). In addition, the D regions of most mouse V_H regions for which amino acid sequences are available contain a tripeptide Tyr-Tyr-Gly that is encoded by the $D_{SP2.1}$ and $D_{SP2.2}$ segments¹¹. Even the D_{Q52} segment which did not cross-hybridize with the $D_{SP2.1}$ -like segments (Y.K., unpublished) shows significant homology, not only in the coding region (Table 1), but also in the flanking regions (Figs 4, 5). Thus, many, if not all D segments are related to the D_{SP2} segments, both evolutionarily and structurally.

Many D regions have extensions on one or both side(s) of the 13-mer (Table 1). The only striking characteristic of the sequences within these extensions is that the 3' extension of the M173-II region shares a heptamer, CGGGGGT, with the H76 D region, the only D region apparently having no homology with the core 13-mer (Table 1). Each of these D regions may be encoded by its own D segment. Alternatively, some D regions, particularly those with relatively long extensions, may be encoded in two or more discrete D segments which are joined in the complete V genes. Two observations argue for the presence of D-D joinings. First, one and four base pairs at the presumed D_{Q52} - J_{H2} joining sites of the B6B1 and Q52 clones were in fact encoded neither by the D_{Q52} nor J_{H2} DNA segment (ref. 19 and Fig. 5). Second, we have been unable to detect a D_{M141} band in Southern gel blots of embryo DNA when this sequence of 44 base pairs (Table 1) was dissected out from the complete V_{M141} gene and used as the hybridization probe. In the same hybridization conditions the J_{H4} band was easily detected using the corresponding probe of nearly the same length (H.S., unpublished). If D-D joinings indeed occur, we predict that some D segments carry putative recognition sequences with 23-base pair spacers on at least one side.

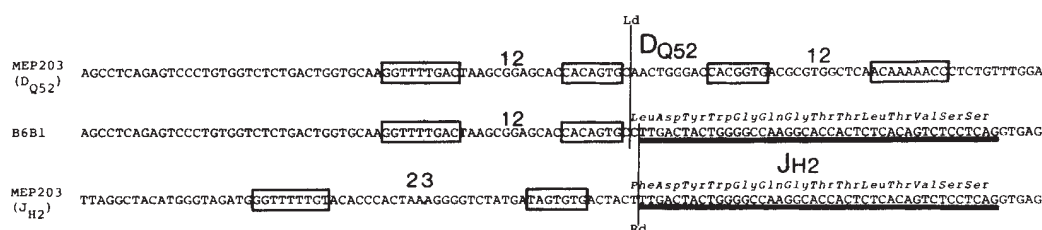
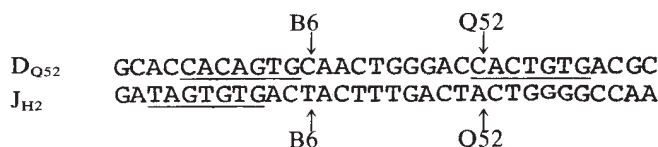


Fig. 5 Nucleotide sequences of the recombination regions of clone MEP203 and clone B6B1. The sequence across the 1.2-kilobase deletion of the B6B1 clone is compared with the two sequences of the embryonic fragment, MEP203, that cover the 5' (top)¹⁹ and the 3' (bottom)⁷ boundaries of the deleted segment. The two vertical lines, Ld and Rd, designate the left (5' side) and the right (3' side) boundaries of the deletion as determined by DNA sequencing, respectively. The other markings are as in Fig. 4.

Somatic diversification of heavy chains by V_H-D-J_H joinings

Amino acid sequence data¹² strongly suggest that the three types of germ-line V gene segments, V_H, D and J_H, combine in various, albeit not all possible, combinations to form complete V genes that are active in myelomas and presumably in B lymphocytes. The presence of the common 'recognition' sequences on the joining ends of all three gene segments supports this 'random' assortment. Furthermore, in both κ light- and heavy-chain genes it has been established that a given J DNA segment can assort with different V DNA segments^{4,5,23}.

Another somatic mechanism that might be used to amplify the coding capacity of immunoglobulin genes has been previously suggested by us⁴ and by Max *et al.*⁵, based on a proposed flexibility in the joining enzyme(s) with respect to the exact recombination sites on a given pair of joining gene segments. Although several previous observations in the κ -gene system support this hypothesis^{4,5,7,24,25}, none of the previous studies provides direct evidence for the hypothesis, because no two joinings examined took place between the same pair of gene segments. The present study, when combined with that described in the accompanying paper¹⁹, presents such a case. Thus, the D-J joinings observed in myeloma QUPC52 and T-cell B6 both used the D_{Q52} and J_{H2}, and yet the exact recombination sites differ both on the D and J sides in the two joinings as shown below,



where the arrows indicate the recombination sites and the underlined sequences are the palindromic heptamers. Provided that these recombinants represent the intermediates of successful V-D-J joinings, we can conclude that modulation of exact recombination sites is another source of antibody diversity that is generated somatically.

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T-cell antigen receptors are not encoded in assembled V_H, D and J_H segments

Our gel-blotting experiments using the J_H hybridization probe demonstrated that the DNA sequences are often, but perhaps more importantly not always, rearranged in the vicinity of the J_H cluster in various clones of cytolytic T cells and in thymomas. Two other groups reported similar findings and implicated them in the somatic formation of the complete structural genes coding for the antigen receptors of T cells^{17,18}. The present results clearly rule out such a simple interpretation of the gel-blotting experiments. The recombinations in both SP2 and B6 cells involve D and J sequences, but no V_H sequence. In fact, neither recombinant can code for a V-like polypeptide chain in the region located 5' to the recombination sites. We also emphasize that the recombination does not always occur among T-cell clones; we observed no indication of rearrangement around the J_H cluster in four well characterized clones of functional cytolytic T cells. We thus conclude that if the V_H DNA pool is shared by T and B cells to encode the antigen receptors, the four J_H segments identified to date are not used for rearrangement, at least in cytolytic T cells. It may be that T-cell receptors are encoded in an entirely different set of genes, or alternatively T and B cells share the same or an overlapping set of germ-line V_H genes, and another set of J-like and C-like DNA segments exists elsewhere in the genome, with which a V DNA segment joins specifically in T cells. Why, then, are there D-J_H joinings in T cells? One possibility is that they occurred in the common precursor cell which gave rise to B and T cells and have no active role in T-cell function. As discussed in the accompanying article¹⁹, D-J_H joinings might be common on the unexpressed copy of chromosome 12 in myelomas, and one recent study²⁶ suggests that this might also be the case in natural B lymphocytes. Alternatively, the hypothetical T-cell recombinase may mediate these joinings due to lack of absolute specificity for the gene segments encoding the T-cell receptors.

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LETTERS

A lower limit for the birth rate of pulsars

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Using experimental data on observed pulsars, we have estimated a lower limit for the birth rate of pulsars in our Galaxy to be $B_{1b} = 0.0111 (\pm 0.0050) K$ pulsars yr^{-1} , where K , the 'beam factor', allows for the possibility that only a fraction of all pulsars is beamed towards the Earth. If we take K to be 5, we obtain a mean birth rate of 1 pulsar per 18 yr while we can place a firmer lower bound with 95% confidence of 1 per 50 yr. Two important new features of the present approach are: (1) we do not assume any model for pulsars; (2) we have allowed for selection effects (in a model independent way). Apart from obtaining a more reliable number for the birth rate of pulsars, we conclude that the quantity $P/(2\dot{P})$, where P is the period of the pulsar and $\dot{P}$ is its time derivative, is probably a good measure of age in 'young' pulsars up to half a million years old, and that selection effects which have been neglected in all earlier calculations are significant. The present calculation reduces the discrepancy between pulsar and supernova birth rates.

We assume that $\dot{P}$ is always positive and that the pulsar distribution in the Galaxy is in a steady state. Let $\rho_i(P, \dot{P}, L) dP d\dot{P} dL$ be the density distribution of pulsars in the Galaxy, where L is the intrinsic pulsar radio luminosity. The distribution $\rho_o(P, \dot{P}, L)$ of pulsars observable from the Earth is then given by

$$\rho_i(P, \dot{P}, L) = KS(L)\rho_o(P, \dot{P}, L) \quad (1)$$

where the scale factor $S(L)$ is the ratio of the total volume of the Galaxy to the volume covered in pulsar searches, both weighted by the known radial density distribution of pulsars in the Galaxy. K is assumed to be independent of P , $\dot{P}$ and L and to have a value¹ 5. Now, the flux or current of pulsars at any period P moving from lower to higher periods is given by

$$\begin{aligned} f(P) &= \int_0^\infty \int_0^\infty \rho_i(P, \dot{P}, L) \dot{P} d\dot{P} dL \\ &= K \int_0^\infty \int_0^\infty S(L) \rho_o(P, \dot{P}, L) \dot{P} d\dot{P} dL \end{aligned} \quad (2)$$

Clearly, $f(P)$ is equal to the number of pulsars being born per unit time in the period $0-P$, minus those dying in the same range. Therefore, $f(P)$ is rigorously less than or equal to the total birth rate B . For better statistics, we consider the following average of $f(P)$

$$F(P_{\max}) = \frac{1}{P_{\max}} \int_0^{P_{\max}} f(P) dP \leq B \quad (3)$$

where the rigorous inequality is obviously true for all values of $P_{\max}$.

We have estimated $F(P_{\max})$ by means of equation (4) using experimental data available for 210 pulsars detected by the Jodrell Bank², Arecibo³ and Second Molonglo⁴ surveys above their quoted minimum flux sensitivities:

$$F_{\text{mean}}(P_{\max}) = \frac{K}{P_{\max}} \sum_i S(L_i) \dot{P}_i; \quad 0 < P_i \leq P_{\max} \quad (4)$$

The quantities $S(L_i)$ were computed using published experimental details regarding sky coverage, receiver temperature, and so on of the above three surveys. As a check on our values of

$S(L_i)$, we have calculated the total number ($\pm$ standard deviation) of pulsars in the Galaxy to be

$$N = K \sum_i S(L_i) = 6.05 (\pm 1.88) \times 10^5 \text{ pulsars} \quad (5)$$

From a careful statistical analysis we have also obtained a lower limit N_{951b} with 95% confidence to be 3.19×10^5 pulsars. These numbers are in good agreement with the standard quoted value^{1,5} of 5×10^5 pulsars. Using equation (4), we have calculated $F_{\text{mean}}(P_{\max})$ and also $F_{951b}(P_{\max})$ at various values of $P_{\max}$ (the solid and dashed lines in Fig. 1). By equation (3), any value of $P_{\max}$ can be selected to set a lower bound on B . However, F_{mean} is sensitive to statistical fluctuations arising from individual pulsars whereas F_{951b} is relatively unaffected. We have therefore selected the $P_{\max}$ at which F_{951b} is maximum. Thus

$$\begin{aligned} B_{1b} &= F_{\text{mean}}(1.3 \text{ s}) \\ &= 0.0555 (\pm 0.0248) \text{ pulsars per yr per Galaxy} \\ &= 1 \text{ pulsar in } 18_{-6}^{+15} \text{ yr} \\ B_{951b} &= 0.0200 \text{ pulsars per yr per Galaxy} \\ &= 1 \text{ pulsar per 50 yr} \end{aligned} \quad (6)$$

The present calculation has two distinct advantages over earlier approaches.

All earlier attempts to estimate B assume^{1,2} that the age τ of a pulsar is given by the quantity $P/(2\dot{P})$. This relation implies that pulsars slow down because of magnetic dipole radiation⁶ and that the magnetic field does not decay. Our calculation has no such model dependent assumptions. However, we have also developed a variant of the τ calculation⁷ using our concept of pulsar current and individual scales, $S(L_i)$. This gives a B_{1b} of 1 pulsar per 27_{-9}^{+29} yr and a B_{951b} of 1 pulsar per 84 yr. From the approximate agreement of these numbers with our rigorous results in equation (6) we conclude that $P/(2\dot{P})$ is a reasonable measure of pulsar age up to about half a million years, thus setting a lower limit on the decay time of the magnetic field.

This is the first calculation to allow for any selection effects which might arise from pulsar luminosities being correlated with P or $\dot{P}$. Such correlations have been neglected in earlier calculations in spite of there being some evidence⁸ for them. To assess the significance of selection effects, we have computed B_{1b} using equation (4) with a single average scale factor $\langle S(L) \rangle$ for all pulsars⁷, thus in essence neglecting correlations between L and P or $\dot{P}$. We then obtain a birth rate of 1 pulsar per

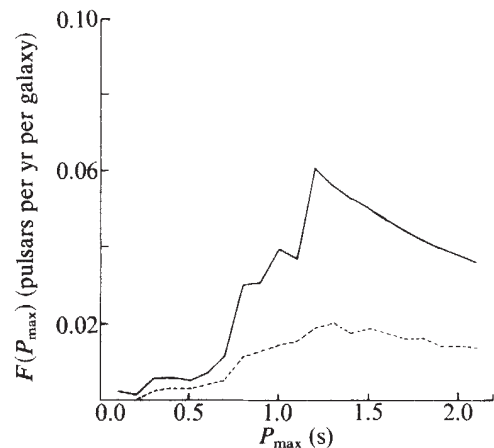


Fig. 1 The solid line shows $F_{\text{mean}}(P_{\max})$ while the dashed line shows $F_{951b}(P_{\max})$. We have selected the values at $P_{\max} = 1.3$ s to estimate pulsar birth rate.

$6.9^{+1.8}_{-1.2}$ yr. From a statistical comparison of this result with that given in equation (6) we can state with greater than 95% confidence that selection effects are significant and cannot be neglected in birth rate calculations. This throws all earlier calculations into doubt.

The present estimate of pulsar birth rate reduces but does not solve a fundamental problem on the origin of pulsars¹. The birth rate of supernovae, commonly believed to be the progenitors of pulsars, has lagged far behind pulsar birth rates, typical estimates ranging from 1 supernova per 50 yr to 1 per 150 yr (ref. 1). Our pulsar birth rate in equation (6) is significantly lower than earlier estimates, which are typically 1 pulsar per 7 yr but has not completely bridged the gap.

After submitting this work we received a preprint by E. S. Phinney and R. D. Blandford in which the concept of a current in the $\dot{P}$ diagram has been used to test pulsar evolutionary models.

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Spin-down episode of the X-ray pulsar Vela X-1

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Since the discovery¹ of a 283-s pulsation of an X-ray binary Vela X-1, several observations^{2–8} between 1975 and 1978 have revealed that its pulse period P was decreasing with an average value of $\dot{P}/P \approx -1.5 \times 10^{-4} \text{ yr}^{-1}$. The long-term spin-up which is considered to be caused by the angular torque of accreting matter, is a common feature of X-ray pulsars. The pulse periods of Vela X-1 were measured by the Japanese satellite Hakucho in March 1979 and March 1980, and the period in the latter was found to be significantly greater than in the earlier period despite the general spin-up between early 1976 and early 1979. Moreover, we have measured for the first time the spin-down rate $\dot{P}/P = (2.6 \pm 0.2) \times 10^{-3} \text{ yr}^{-1}$ in March 1980. This spin-down phenomenon is of great importance, as it is considered to be a manifestation of the dynamical behaviour caused by the internal structure of a neutron star.

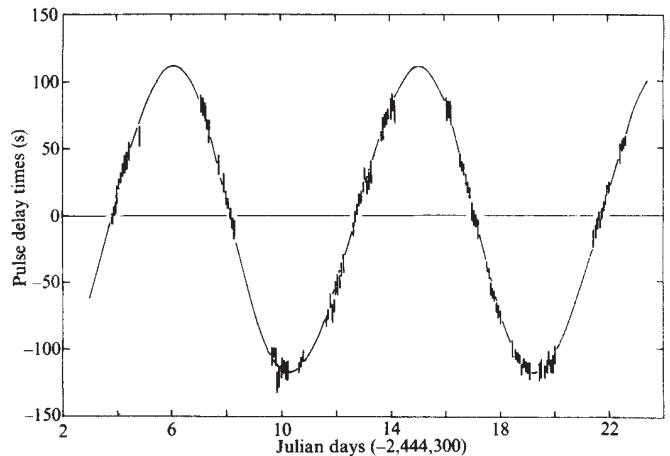


Fig. 1 Doppler delay curve of the Vela X-1 pulse arrival times observed in March 1980 compared with the calculated delay curve for the best-fit eccentric orbit with parameters given in Table 1. The vertical bars are measured delays with the length representing $\pm 1\sigma$ error limits.

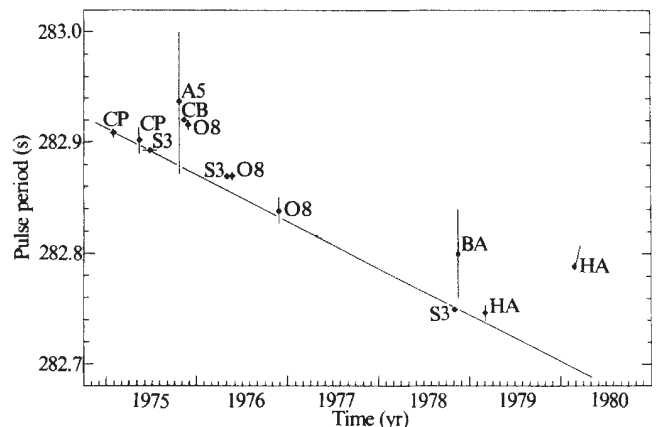


Fig. 2 The long-term pulse period history of Vela X-1. The measurements were made with Copernicus and Ariel V (CP and A5)⁶ SAS 3 (S3)^{2,4,7}, COS B (CB)³, OSO 8 (O8)⁵, Balloon (BA)⁸ and Hakucho (HA, present work). The solid line represents a long term spin-up at a rate of $\dot{P}/P = -1.5 \times 10^{-4} \text{ yr}^{-1}$ for guiding eyes. The short solid line attached to the 1980 point indicates the spin-down rate derived from the present observation (see text).

We observed Vela X-1 in 8.1–21.8 March (UT) 1979 and in 5.2–24.0 March (UT) 1980 from Hakucho, with its spin axis detectors in the energy range 1–22 keV. The binary period, the duration of eclipse, the pulse profile and its energy dependence are in good agreement with earlier results. The X-ray flux varied erratically by a factor of four in positive correlation with the softness ratio, which is the ratio of counting rate in the energy range 1–9 keV to that in range 9–22 keV, in addition to the variation associated with the binary phase. The X-ray luminosity observed in March 1980 was $(1-5) \times 10^{35} \text{ erg s}^{-1}$ in the energy range 2–22 keV at the estimated distance of 1.2 kpc, which is comparable with that in the previous observations.

Despite the differences in profile and intensity of each pulse the epoch of the pulse arrival time can always be identified by the sharp minimum of the pulse profile. Its epoch is obtained only within an accuracy of ~ 10 s for each pulse. Utilizing an uninterrupted pulse train consisting of several successive pulses for each satellite orbit improves the accuracy to 3 s. We obtained 35 and 115 epochs of pulses in 1979 and 1980, respectively.

Table 1 Best-fit orbital parameters for Vela X-1

Parameters	SAS 3 [†] November 1978	Hakucho March 1979	Hakucho March 1980
$a_x \sin i (l-r)$	112.3 ± 0.8	113.4 ± 3.8	114.4 ± 1.3
$f(M) (M_\odot)$	19.0 ± 0.4	19.5 ± 2.1	20.0 ± 0.7
e	0.094 ± 0.005	0.04 ± 0.07	0.091 ± 0.019
ω	$158^\circ \pm 5^\circ$	$182^\circ \pm 26^\circ$	$160^\circ \pm 7^\circ$
$P(s)$	282.7468 ± 0.0004	282.746 ± 0.006	282.7874 ± 0.0014
$\dot{P}$	$(-9 \pm 18) \times 10^{-9}$	$(-20 \pm 10) \times 10^{-9}$	$(23.0 \pm 1.8) \times 10^{-9}$
$\tau(\text{JD} - 2,440,000)$	$3,823.53 \pm 0.13$	$3,938.4 \pm 0.7$	$4,307.68 \pm 0.18$
$P_{\text{orb}}(\text{days})$	$8.9643\$$	$8.965\$$	$8.965\$$

*All quoted uncertainties are single parameter 1σ confidence limits.

[†]After Table 1 of ref. 7

[‡]Corresponding to $\dot{P}/P = (-0.001 \pm 0.002) \text{ yr}^{-1}$.

[§]Orbital periods are fixed in the fitting.

The epoch of the n th pulse, after correcting for the heliocentric motion of the Earth, is expressed as

$$t_n = t_0 + P_0 n + \frac{1}{2} P_0 \dot{P} n^2 + a_x \sin i F(\theta, \omega, e, \tau)$$

where P_0 is the pulse period at a specified epoch t_0 , $\dot{P}$ is the rate of change of the period, $a_x \sin i$ is the projected semi-major axis of the orbit of the X-ray star, and F represents the effect of an eccentric motion with the periastron longitude ω , the eccentricity e , the time of periastron passage τ , and the mean anomaly $\theta = 2\pi(t - \tau)/P_{\text{orb}}$. The orbital period derived from X-ray eclipses between November 1978⁷ and March 1980 is $P_{\text{orb}} = 8.965 \pm 0.001$ days. This is considered a fixed parameter for the present analysis. Table 1 shows the orbital parameters obtained by fitting the observed pulse arrival times to the above equation. The best-fit Doppler curve for the data of March 1980 is shown in Fig. 1. All the parameters except P and $\dot{P}$ are consistent with earlier results^{2,3,7}.

Both the increase of the pulse period P between March 1979 and March 1980 and the positive value of $\dot{P}$ in March 1980 are remarkable. In March 1979 $P = 282.745 \pm 0.006$ (1σ)s, which lies nearly on the extrapolated line with $\dot{P}/P = -1.5 \times 10^{-4} \text{ yr}^{-1}$, whereas $P = 282.7874 \pm 0.0014$ (1σ)s in March 1980 which is significantly above this line, as shown in Fig. 2. This appreciable change in P seems to indicate a spin-down of the pulsar within a year. A similar increase of P was observed in late 1975³ and is suspected to be associated with a flare occurring in August 1975⁹. The increase in period observed in March 1980 may also be related to a flare in July 1979 observed by Hakucho¹⁰. Utilizing the data over two orbital cycles in March 1980 we obtain $\dot{P} = (23.0 \pm 1.8) \times 10^{-9}$, or $\dot{P}/P = (2.6 \pm 0.2) \times 10^{-3} \text{ yr}^{-1}$. Thus Vela X-1 occasionally undergoes a spin-down in the course of spin-up over a period of many years.

The observed increase of spin period can hardly be explained by fluctuations in the accretion rate^{11,12}, because the X-ray intensity in March 1980 is essentially the same as it was previously, although the model gives a favourable explanation for the period changes of the fast rotator such as Her X-1 and Cen X-3. Other external mechanisms of torque variation, such as mass ejection from the neutron star or the reversal of accreting stream in the wind-fed accretion, seem to have difficulty in accounting for the observed high rate of change of the pulse period. The internal torque acting on the crust of the neutron star, such as the braking stress of the rotation of crust caused by a lagging fluid core¹³, seems to be a possible explanation of the observed spin-down episode. A detailed consideration the theoretical treatment of such a problem as well as further observations of the period change will be reported elsewhere.

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The very soft X-ray transient H1853+60

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The increased sensitivity, sky coverage and spectral range of X-ray observatories have revealed a wide range of transient phenomena. The 'classical' X-ray transients flare rapidly, sometimes increasing by more than an order of magnitude in X-ray flux in a matter of days, and decay on time scales of months. Such transients are mainly located in the galactic plane, often associated with 'steady' X-ray sources¹. Some high galactic latitude transients, most of which are optically unidentified, have been observed to vary on time scales of tens of seconds to days^{2,3}. Most transient sources have been detected at energies greater than 1 keV; more recently, however, softer X-ray transients have been detected with observations on HEAO 1 and other satellites⁴⁻⁹. We now report detection of a new soft X-ray transient, H1853+60, using data from the low energy detectors (LEDs) on the HEAO 1 A-2 experiment.

H1853+60 was observed by both LEDs 1 and 2 during a series of scans in ecliptic latitude on 13 May 1978 (see ref. 8 for details of the A-2 experiment operation). The source was detected at levels of 5.3 and 6.3 σ above background on two scans by LED 1 in the 0.16–0.4 keV band. There is no evidence for any emission above 0.5 keV. The source detection in LED 2 at the same sky location as LED 1 is significant, since that detector is offset by 6° from LED 1 in the scan plane, providing convincing evidence that the transient was not due to time-dependent particle events or instrumental effects. The source is unresolved by the 1.55° FWHM field of view of LED 1 along the scan direction.

Figure 1 plots the light curve of H1853+60 in the 0.16–0.4 keV band for 30 April–23 May 1978 (days 120–143). The source intensity has been corrected for the LED 1 collimator response (2.95° FWHM) perpendicular to the scan direction, assuming that the true source location is at the centre of the 90% confidence error box shown in Fig. 2. Scans with angular offsets $\geq 1.5^\circ$ from scan plane passing through the error box centre were not included so as to minimize possible systematic errors. Before 10 May, there is no evidence for a steady source (2σ upper limit ≈ 1 c.p.s.); an upper limit to flare-like behaviour is ≤ 3 c.p.s. From 11 to 16 May, the data are inconsistent (probability $\leq 1\%$) with any single count rate, even if we remove the two $\geq 5\sigma$ 'flare' scans. Summing scans together on a day-to-day basis, we also find no evidence for the presence of a source ($\leq 2\sigma$) before 10 May, but there is some ($\sim 3.5\sigma$) indication of post-outburst emission on 14 May; by 15 May the source is gone.

The peak LED 1 count rate on 13 May was 9.3 ± 1.5 c.p.s. above background, corresponding to a broad-band flux of $\sim 3 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$ in the 0.16–0.4 keV band. H1853+60 was not detected in a steady or flaring state 6 months earlier when HEAO 1 scanned the same area of the sky. In addition, no steady source was detected in the HEAO 1 MED or HEDs (2–10 keV band)¹⁰, and an examination of individual MED and

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HED scans while H1853+60 was flaring shows no evidence for X-ray emission (F. Marshall, personal communication).

Because of the relatively poor spectral resolution of proportional counters at such low energies, we can only obtain upper limits to the source temperature and interstellar medium column density of $T \leq 10^6$ K and $N_H \leq 10^{20.6} \text{ cm}^{-2}$, respectively. The spectrum does, however, exhibit a steep rise at the lowest detectable energies, suggesting that much of the energy output of the transient event was below our detection threshold of 0.16 keV.

A search for possible optical counterparts for H1853+60 in the available literature has produced the following candidates: the Mira variable AD Dra which is located at the northeastern corner of the error box, the faint (15th magnitude) galaxy MCG+10-27-001, the centre of the Zwicky cluster ZC1851.3+6032, the nearby double star Gliese 725A and B, and a number of 8th and 9th mag SAO stars. There are no previously discovered X-ray sources, Yale catalogue bright stars, RS CVn systems and other variable stars, white dwarfs, or other proper motion or nearby stars in the error box.

Gliese 725AB (dM4, dM4.5; HD173739) has been reported as a flare star¹¹. Although there is no evidence for hydrogen emission—a characteristic usually associated with UV Ceti flare stars—Ca II emission has been observed (this, however, is common in late-type dwarfs)¹². Few X-ray flares from UV Ceti stars have been observed, and those that have been are generally seen at harder (≥ 2 keV) energies than H1853+60 (refs 13, 14). AT Mic, which was detected by both the HEAO 1 LEDs and MED, had a flaring spectrum characterized by $T \geq 10^7$ K (ref. 14). The detection of H1853+60 at $\geq 5\sigma$ for two successive scans separated by 0.1 day is also not consistent with typical UV Ceti flare risetimes¹³. If HD173739 proves to be the optical counterpart of H1853+60, one possibility is that we have observed a 'long-duration enhancement' such as that seen in the solar corona, with risetimes of the order of hours, and decay times of a day or more; even these enhancements, however, have characteristic temperatures only slightly lower ($\sim 3\text{--}7 \times 10^6$ K) than those of a solar flare^{15,16}.

Although the stellar flare hypothesis cannot be definitely ruled out, we must also consider the similarity of H1853+60's spectral and temporal characteristics to several other transients observed by the HEAO 1 LEDs^{8,9}, suggesting a common emission mechanism for these events. As the transients referred to were initially discovered using 1-day superpositions of the LED data, which have been examined extensively (J. Nousek, personal communication), it is unlikely that many more transients as strong as these will be discovered, except on an individual scan basis. The only unusual class of optical object common to the transients' error boxes are Mira variables, of which $\sim 4,500$ are known. Given an error box area of $\sim 1.5 \text{ deg}^2$,

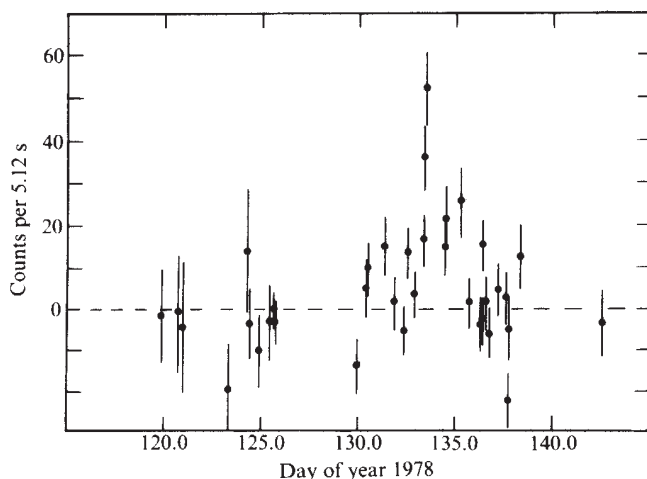


Fig. 1 Light curve of H1853+60 in the 0.16–0.4 keV band. Error bars are $\pm 1\sigma$.

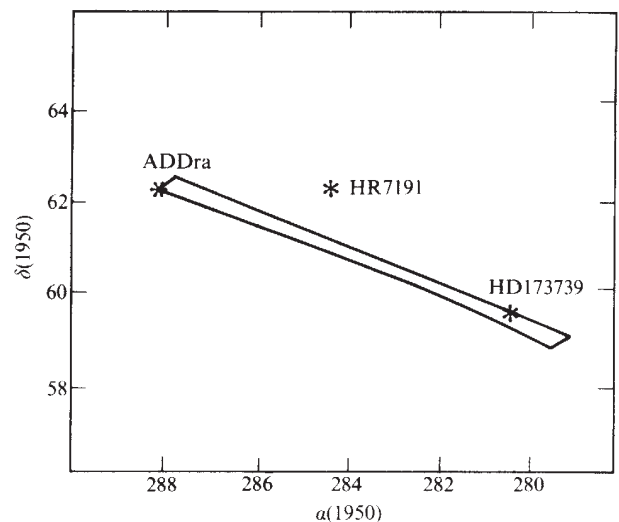


Fig. 2 90% confidence error box for H1853+60; HD173739 = GL725A.

there is $\geq 10\%$ probability that a Mira variable would be found within a typical LED error box by chance alone. (Ref. 8 reviews the possibility of such transient X-ray emission in supernova outbursts, undiscovered RS CVn systems and shock heating of the atmospheres of the Mira variables described above; none of these mechanisms is likely to account for emission in H1853+60.)

The suggestion of a U Gem-like outburst from an as yet undiscovered dwarf nova (also discussed in ref. 8) is intriguing because of the similarity of the spectra observed in both U Gem⁵ and H1853+60, and the approximately equal risetimes for the outbursts. Although U Gem was also observed in harder (2–10 keV) X rays¹⁷, the 0.16–0.4 keV peak flux from H1853+60 is about 10 times less than that from U Gem, and thus if the harder X rays scale proportionately, the hard X-ray flux from such a dwarf nova outburst would fall below the sensitivity level of the MED or HEDs for single scans ($\sim 4\text{--}6 \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$). If we assume that H1853+60 is indeed a dwarf nova with the same X-ray luminosity as U Gem, we derive a distance of about three times that of U Gem, or ~ 300 pc, based on usual distance estimates⁵. If the optical outburst is scaled in luminosity, the optical counterpart of H1853+60 would be at $V \sim 12$ at outburst and $V \sim 16$ when quiescent. Although fainter U Gem-type systems have been discovered¹⁸, the completeness of the sample of U Gem stars down to a particular limiting magnitude is unknown; further optical observations will be needed to confirm the dwarf nova hypothesis.

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The Kelvin equation and the capillary condensation of water

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The Kelvin equation¹ relates the equilibrium vapour pressure of a liquid to the curvature of the liquid-vapour interface. It predicts that undersaturated vapours will condense in channels of sufficiently small dimensions. While the applicability of the Kelvin equation to organic liquids with meniscus radii as low as 4 nm has been verified by direct experiment², its applicability to water for meniscus radii below 4 nm is unverified, and has been doubted^{3,4}. We have used Fizeau interferometry to measure directly the capillary condensation of water in a wedge between fused silica surfaces. Measurements were made at relative vapour pressures (P/P_s) from 0.996 down to 0.945, corresponding to theoretical meniscus radii from ~120 to 9 nm. We find only a small discrepancy between our experimental results and the Kelvin equation in the vapour pressure range covered, provided that correct account is taken of the effect of the adsorbed film, up to 200 nm thick, on the meniscus shape.

The Kelvin equation forms the basis of critical nucleation theory⁵, and is widely used in the interpretation of such diverse phenomena as adhesion⁶, the enhanced solubility of small particles⁷ and the retention and transport of liquids in porous materials⁸⁻¹⁰. Because water is an important liquid in the above applications, we need direct experimental evidence concerning its capillary condensation behaviour.

Cross and Picknett¹¹ have shown experimentally that water obeys the Kelvin equation for meniscus radii $> \sim 4 \mu\text{m}$, but most applications of the equation involve much smaller radii. Other experiments^{3,4} have purported to show that the Kelvin equation grossly underestimates the degree of capillary condensation of water, but these results are now generally ascribed to the presence of impurities^{4,12}, and there have been no reliable studies in the important region covering menisci with radii $< 1 \mu\text{m}$.

For the present experiments, two silica surfaces (either two optical flats held as a very narrow wedge, or a spherical surface of radius 69 m on a flat surface) were clamped between Teflon supports in an evacuated isothermal enclosure. The vapour pressure in the enclosure was controlled by an aqueous potassium nitrate solution. Stringent cleaning procedures, including prolonged boiling in nitric acid followed by prolonged boiling in freshly distilled water and finally blowing dry with filtered dry nitrogen, were applied to the surfaces.

Each silica surface was first tested for wettability by exposing it to the vapour above warm distilled water and observing the appearance of coloured interference fringes on the surface (the so-called 'steam test')¹³. Surfaces for which the contact angle is non-zero exhibit hazy condensation due to microdroplets, rather than clear, broad fringes. At the end of an experimental run, the silica surfaces were carefully separated so that the condensed liquid collected at one point, where it evaporated. The surfaces were then examined in a strong light to ensure the absence of any residue. This test is sufficiently sensitive to detect dissolved material equivalent to 0.01 of a monolayer. The surfaces were then retested for wettability, and finally atomic absorption spectroscopy was used to test for the presence of inorganic ions (for example, K^+) in a small volume (0.1–0.2 ml) of water rinsed over each surface after each experiment. Only if all these tests were passed was the result of an experiment accepted. Also, no experimental run was accepted unless the same results were obtained with increasing and decreasing relative vapour pressure.

The appearance of capillary-condensed water in a wedge between two optical flats is shown in Fig. 1. The Fizeau fringes are shifted with respect to their position when the surfaces were dry because of the presence of the adsorbed water film. This shift was used to calculate the adsorbed film thickness Z_1 (Table 1). The distance D_{men} between the silica surfaces at the meniscus can then be calculated from the fringe positions in the dry state. The results of condensation experiments for both the wedge and the sphere-on-flat configuration are given in Fig. 2, in which $-\ln(P/P_s)$ is plotted against $1/D_{\text{men}}$.

The finding that water reversibly forms relatively thick adsorbed films on a fused quartz surface agrees with the results of Pashley and Kitchener's¹³ ellipsometric study. These results clearly have a bearing on the question of water 'structure' at solid interfaces^{4,12,13}, but we cannot add any comment to that already made by Pashley and Kitchener who have shown that very thick films are consistent with calculations from double layer repulsion theory. However, several authors have postulated deviations from the Kelvin equation because of the influence of such a thick adsorbed film (more correctly, the influence of a long-range adsorption potential) on meniscus curvature¹⁴⁻¹⁷, and it is with this aspect that we are now concerned.

All authors find the same form for the Kelvin equation for the case of capillary condensation in a slit-shaped pore^{17,18}:

$$D_{\text{men}} = \frac{2\gamma V_m}{R_0 T \ln(P'/P_s)} + 2Z_1 - \left\{ 2/R_0 T \ln(P'/P_s) \int_{Z_1}^{\frac{1}{2}D_{\text{men}}} F(Z) dZ \right\} \quad (1)$$

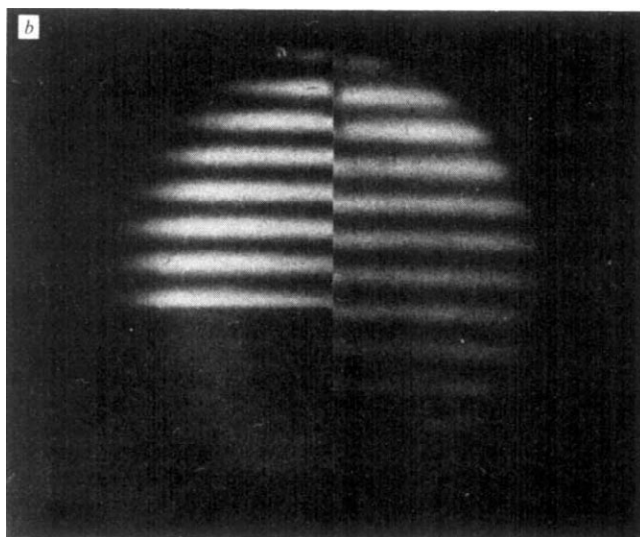
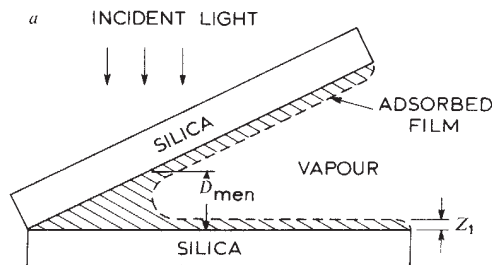


Fig. 1 *a*, Diagrammatic representation of liquid condensed in wedge between two optical flats. *b*, Comparison of appearance of wedge, viewed normally in monochromatic light ($\lambda = 546.1 \text{ nm}$) before (right-hand side) and after (left-hand side) exposure to water vapour at $P/P_s = 0.9969$. Note that all fringes have shifted equally, showing that the Laplace pressure in the condensed liquid does not distort the silica surfaces. Silica surfaces are in contact at the bottom of the picture, the bright mark at the top is a gold foil spacer.

Table 1 Experimental adsorbed film thickness Z_1 of water condensed in a wedge between flat silica surfaces

Relative vapour pressure P/P_s	Z_1 (nm)	$-0.43/\ln(P/P_s)$ (nm)
0.90	3*	4.1
0.95	5*	8.9
0.965	8*	12.0
0.98	20, 27*	21
0.9925	(59 ± 5)†	57
0.9944	(82 ± 5)†	77
0.9955	(120 ± 10)†	95
0.9969	(200 ± 30)†	139
1.00 ± 0.001	150*‡	∞

* Data from ref. 13: we obtained agreement with these figures in an experiment where the condensation was reversible, but the results were rejected because of trace quantities of residue at the end of the run.

† Present work.

‡ Agrees with calculations based on double-layer theory.

where γ is the surface tension, V_m the molar volume, P' is the vapour pressure at which the liquid just irreversibly evaporates, Z the normal distance from the solid surface, R_0 the gas constant and T the absolute temperature (this form of the equation neglects corrections which may be necessary at extremely high meniscus curvatures¹⁹). The first term is the simple Kelvin term, the second makes allowance for the adsorbed film by simple subtraction, and the third is a correction factor to allow for the effect of adsorption potential on meniscus curvature. The function $F(Z)$ is the adsorption isotherm of water on a flat surface. The accuracy of the adsorption data does not warrant numerical evaluation of this integral over the whole meniscus, but fortunately the simple expression Z_1 (nm) = $0.43/\ln(P_s/P)$ follows the data sufficiently closely over much of the range of interest, so $F(Z)$ can be put in the form $F(Z) = 0.43R_0T/Z$, the integral of which can be obtained by a simple iterative method¹⁷.

The uncorrected Kelvin equation, the Kelvin equation with allowance for the adsorbed film by simple subtraction, and equation (1) are plotted in Fig. 2. Only equation (1) is in reasonable agreement with the experimental data, although there is still a significant discrepancy. This discrepancy may well be due to the breakdown of the 'boundary layer approximation' implicit in equation (1). That is, it may not be possible to treat

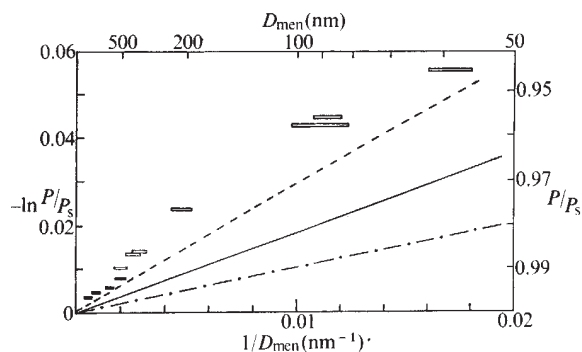


Fig. 2 Experimental results for capillary condensation of water in a wedge between silica optical flats (closed bars) and in the gap between a spherical silica surface and contacting silica optical flat (open bars). The size of the rectangles represents standard errors, based on at least three measurements of the position of each dark fringe. —, Uncorrected Kelvin equation, first term of equation (1). —, Kelvin equation with correction by subtraction for thickness of adsorbed film, first two terms of equation (1). - · - ·, Fully corrected Kelvin equation (1). —, Experimental results from wedge configuration. —, Experimental results from sphere-on-flat configuration. For the sphere-on flat experiments, values of Z_1 were calculated from Z_1 (nm) = $-0.43/\ln(P/P_s)$.

the adsorption and capillary potentials as strictly additive when Z_1 is of the order of $D_{men}/2$. This point has been discussed in detail elsewhere²⁰. The fact that the difference between theory and experiment apparently decreases with increasing D_{men} supports this interpretation.

We conclude that water reversibly forms relatively thick adsorbed films (up to 200 nm) on fused silica surfaces. The origin of the stability of these films is not yet fully understood¹³. The presence of these films affects the meniscus shape for capillary-condensed water, and this effect accounts almost entirely for the capillary condensation behaviour of water. The small residual difference between theory and experiment may well be a consequence of the breakdown of the 'boundary-layer' approximation used in current theories. A fuller report of this work will be given elsewhere.

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Hydrogen production from photolysis of steam adsorbed onto platinized SrTiO₃

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Photodissociation of high pressure (1–8 atm) water vapour adsorbed onto the partially platinized surface of single crystal SrTiO₃ has been observed. The reaction is catalytic; several thousands of monolayers of hydrogen have been produced. The reaction requires light of greater than bandgap (3.1 eV) energy, and proceeds vigorously only if elevated temperatures (375–450 K) and high pressures are used. Recombination of products and poisoning of the metal apparently diminishes the reaction rate after several hours. Previous work on this reaction has been done in the presence of an electrolyte, generally at room temperature. The present work is a significant departure from such an environment, and is important because the surface is not contaminated with an electrolyte. The surface is therefore accessible to ultrahigh vacuum surface analytical techniques such as electron spectroscopy, before and after the reaction. From these studies detailed information about the mechanism may be obtained. In addition, the existence of a surface hydrogen species at elevated temperatures allows us to combine water photolysis directly with other catalytic reactions, such as ammonia synthesis with N₂ and methane synthesis with CO or CO₂.

A reaction chamber made of stainless steel was fitted with a sapphire window and placed in the oven of a gas chromatograph. Light from a high-pressure mercury discharge lamp passed through the window and was incident on the surface of the crystal. The intensity was $\sim 10^{16}$ photons $\text{cm}^{-2} \text{s}^{-1}$ in the energy range above the band gap. A single crystal wafer of SrTiO_3 with nominal (111) orientation was rough-polished and coated *in vacuo* with platinum evaporated from a tungsten filament. We used non-conducting, stoichiometric SrTiO_3 as bulk electrical conductivity has little influence on localized surface reactions. The roughness of the surface ensured that it was only partially covered with platinum. The photoreaction rate was highest with a transparent coating of platinum and decreased with heavier, more opaque coatings, which would cover all semiconductor surface sites.

Ultrapure water was admitted into the evacuated cell at the beginning of an experiment, and pumped through a loop which included the cell and an automatic sampling valve, so that gas samples could be withdrawn periodically. A sample was then analysed in the gas chromatograph, which was fitted with a thermal conductivity detector. With argon carrier gas, we could detect 10 monolayers of H_2 easily, and O_2 with about 20 times less sensitivity. The whole system accessible to water vapour was kept at uniform temperature so that high pressures could be achieved. Figure 1 shows the cell design.

Figure 2 shows H_2 accumulation curves as a function of time for different temperatures, where the saturation vapour pressure of water was used in each case. To inhibit the recombination of H_2 and O_2 products, a few grammes of 0.025 mm Cu foil was placed in the reaction cell to getter the O_2 present. Without this extra copper to oxidize, and thus remove active O_2 from the system, less than half the H_2 would be detected. Oxidation of Cu by water with a release of H_2 was ruled out by blank experiments. Thus the recombination of hydrogen and oxygen is a significant process in these conditions. This is not surprising in view of the presence of platinum and a light activated semiconductor, either of which might act as a catalyst. Because of this back reaction, O_2 was not detected. Blank experiments showed no hydrogen production without platinum on the crystal, nor with platinum on an alumina substrate, and very poor production when platinum was deposited on the dark side of the crystal.

The curves in Fig. 2 show an induction period which depends on exposure to water vapour. If the crystal is exposed to water vapour in the dark for 1–2 h, hydrogen production will proceed immediately on illumination. However, when the crystal is illuminated *in vacuo* for the same period and then exposed to water vapour, the induction period is observed. Thus there is

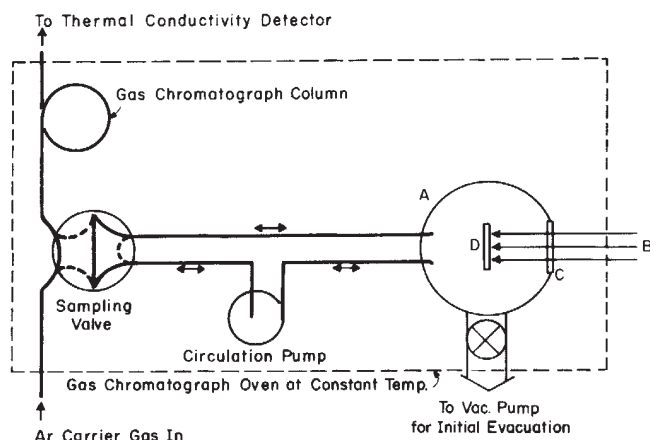


Fig. 1 Apparatus: A, stainless steel cell; B, UV light from Hg lamp; C, sapphire window; D, SrTiO_3 crystal with Pt on the side facing the light. Water vapour circulates through cell, pump, and sampling valve.

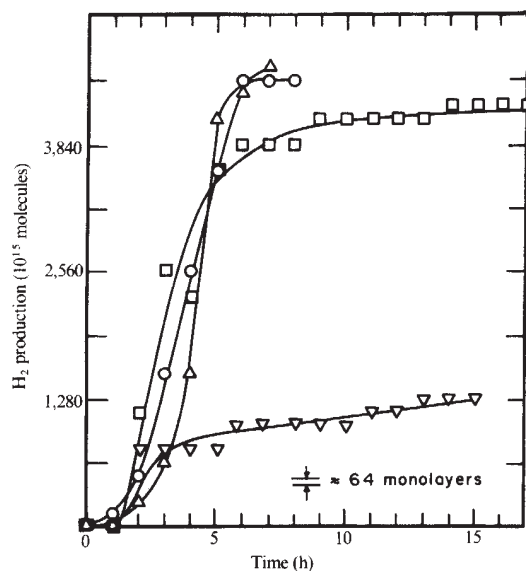


Fig. 2 H_2 product accumulation for four different temperatures at H_2O saturation vapour pressures. Δ , 160°C, 6 atm; $\circ$, 140°C, 3.5 atm; $\square$, 120°C, 2 atm; ∇ , 100°C, 1 atm.

evidence for a slow surface reaction, such as hydroxylation or displacement, which seems to be a necessary precursor for the efficient catalytic photolysis of water observed after the induction period. During efficient hydrogen production, a rate of as much as 500 monolayers per hour has been observed. Within ~ 10 h, however, the hydrogen production rate diminishes. Besides equilibration of the production and recombination rates there is also a surface poisoning effect which contributes to this decline. If a crystal used in the experiment is not regenerated by replacement of the platinum coating, it will yield much less hydrogen in a subsequent experiment. A similar deactivation was noted by Wrighton *et al.*¹ in the SrTiO_3 -Pt electrochemical cell.

It was possible to carry out the photolysis of water at less than saturation vapour pressure. At constant pressure, the reaction rate declines with increasing temperature, as expected, because the recombination over platinum is more facile at higher temperatures. At constant temperature, the rate of hydrogen production increases with increasing pressure.

Recently we reported the photocatalysed dissociation of water vapour on platinum coated and metal-free SrTiO_3 crystal surfaces which were covered with a film of NaOH (refs 2, 3). The need for NaOH was traced to the importance of hydroxylation of the oxide surface. In the conditions of the present experiment the surface is expected to be hydroxylated⁴, and this effect is apparently equivalent functionally to the effect of NaOH.

In conclusion, we have observed evidence for the following steps during photolysis of water on platinized SrTiO_3 : (1) generation of electron-hole pairs by light of greater than bandgap energy; (2) dissociative chemisorption of water vapour on the hydroxylated semiconductor surface; (3) diffusion of a surface hydrogen species to platinum islands and desorption of hydrogen from the islands; and (4) recombination of hydrogen and oxygen which limits the amount of hydrogen that can be generated in a batch reactor.

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Fluid inclusion Rb–Sr isochrons for dating mineral deposits

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The precise dating of mineral deposits has important implications for understanding the lifetime of ore-forming systems and their temporal relationship to various geological events. Not all deposits provide material suitable for conventional radiometric dating, or data which are amenable to unequivocal interpretation. Nor is it easy to verify that such samples have remained isotopically 'closed' since their formation. Fluid inclusions provide an alternative 'closed system' approach. By analysing the isotopic composition of Rb and Sr in inclusions within quartz from the Carrock Fell tungsten vein deposits, Cumbria, we demonstrate here the feasibility of obtaining Rb–Sr fluid inclusion isochrons. The age for the mineralization 392 ± 5 Myr is in good agreement with the mean K–Ar age 387 ± 6 Myr for the vein micas, and confirms the significance of fluid inclusions as precise Rb–Sr geochronometers.

Fluid inclusions are small, usually microscopic, volumes of fluid trapped within crystals during their growth from the fluid. To be used as geochronometers their origin must be known and also that nothing has been added or lost from the inclusion after sealing. The inclusions have usually been trapped at more than one stage in the growth and subsequent fracturing and healing of the crystal. For the purposes of dating, however, as long as the various inclusions were formed in a relatively short time interval, the crystal can be treated as a single temporal event. For a more complex chronology, samples representing individual episodes of mineral deposition can be selected.

In most geological conditions inclusions do not leak¹ although exceptions are excessive overheating² and the development of an intracrystalline deformation fabric³ within the host mineral. The former leads to thermal rupture with a complete emptying of the inclusion, whereas the latter may vary from a simple 'drawing-out' of the inclusion with minor leakage, to total rupture and destruction. Host minerals with little or no cleavage and good mechanical strength are thus preferred. Quartz, a ubiquitous mineral of hydrothermal ore deposits is suitable, particularly as its high chemical purity permits analysis of the inclusion contents independently of ion impurities in the host crystal.

Inclusions provide a means of applying basic geochronological concepts to situations where conventional mineral and rock studies have failed. Excluding fission track dating, the use of inclusions as geochronometers has the same restrictions as all radiometric methods of dating.

(1) The mineral or rock samples must have behaved as 'closed' systems with respect to the parent–daughter isotopes according to the equation

$$D = D_0 + P(e^{\lambda t} - 1)$$

where D is the total number of daughter atoms present in the system; D_0 the number of daughter atoms initially present; P , the number of parent atoms remaining in the system; λ , the decay constant for the parent radionuclide; and t , the time elapsed ('age' of the system). (Inclusions in transparent minerals can be checked before analysis to ascertain whether thermal

rupture or intracrystalline deformation has caused them to leak.)

(2) The mineral or rock samples were formed in a time interval that was short compared with their age. (Modern hydrothermal-hot spring systems driven by local igneous heat sources have lifetimes of a few thousand to approximately one million years. Presumably palaeohydrothermal systems associated with igneous rocks had similar lifetimes. Hydrothermal ores precipitated on the sea floor are thought to have had formation times of a few thousand years because they contain little detritus, even though the rocks above and below are normal detrital sediments⁴.)

(3) A value for the initial number of daughter atoms (D_0) can be assumed or calculated.

Inclusions in quartz can be thought of as droplets of fluid preserved, totally isolated from their external environment, and perfect for Rb–Sr dating. The only disadvantage is their small size, normally $<20 \mu\text{m}$ in diameter. For a fluid containing 1,000 p.p.m. Sr a single inclusion may provide less than 10^{-12} g Sr for analysis.

To test the feasibility of using fluid inclusions as Rb–Sr geochronometers for ore deposits we have taken samples from the tungsten deposits at Carrock Fell, Cumbria. These deposits are characterized by a series of steeply dipping north–south wolframite-bearing quartz veins developed at the contact of the Caledonian Skiddaw Granite with the Lower Palaeozoic Skiddaw Slate Series. Mineral paragenesis studies^{5,6} suggest a main period of hydrothermal activity characterized by continuous quartz deposition, with wolframite and apatite confined almost exclusively to the earliest generations of quartz. Much of the quartz is therefore economically 'barren'. Late-stage hydrothermal activity is represented by a network of cross-cutting east–west carbonate veins and stringers, some of which cement and infill vuggy parts of the quartz veins. Compared with the tin–tungsten deposits of south-west England, the mineralization constitutes a simpler hydrothermal event, which with data from a previous geochronological study⁵ was ideal for investigation. The samples were selected from an extensive suite of vein quartz material collected from underground workings at the Carrock Fell Mine and were representative of both the early and late phases of quartz deposition. The quartz contains abundant two-phase fluid inclusions consisting of a liquid phase and complementary vapour phase, the latter occupying $<25\%$ of the total inclusion volume. They average $5\text{--}30 \mu\text{m}$ in diameter, are ovoid or multifaceted in form, and occur in both isolated non-planar groups and discontinuous intersecting planar arrays. Genetically the inclusions are primary, but because it is difficult to relate them to obvious growth features they are best described as pseudosecondary^{7,8} (they correspond to fluids trapped along microfractures during crystal growth). Collectively the inclusions can be treated as the product of a single mineralizing

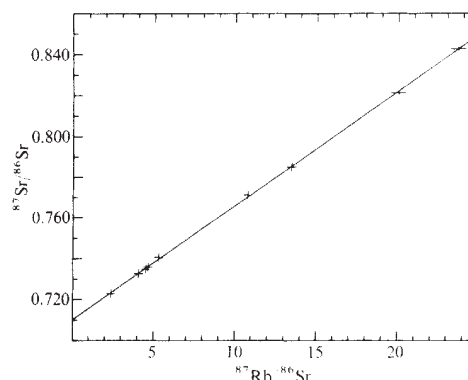


Fig. 1 Fluid inclusion Rb–Sr diagram. Age, 393 ± 5 Myr; intercept, 0.70997 ± 0.0004 ; MSWD, 1.42.

Table 1 Rb–Sr data for inclusion fluids, Carrock fell tungsten deposit, Cumbria

Sample no.	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$
CF-79-10	446	287	4.511	0.73465
	512	281	5.289	0.74043
	475	298	4.625	0.73577
	388	278	4.041	0.73239
CF-79-21	1,225	179	20.01	0.82071
	1,477	183	23.69	0.84253
	505	164	8.971	0.75892
CF-79-30A	166	207	2.318	0.72296
CF-79-30B	765	166	13.46	0.78496
	1,112	301	10.77	0.77102

event. Unlike the pseudosecondaries, secondary inclusions are less well developed and clearly associated with the late-stage carbonate fluids. They can be recognized by their distinctive chemistry and behaviour during low temperature microthermometric studies. Samples suspected of containing either secondaries or inclusions which had leaked were rejected.

The sample preparation and mass spectrometric analysis will be described elsewhere. Conventional isotope dilution techniques⁹, modified to accommodate low level, small samples, were used (the results, given in Table 1, are plotted in Fig. 1). Analytical errors ($\pm 1\%$ $^{87}\text{Rb}/^{86}\text{Sr}$; $\pm 0.03\%$ $^{87}\text{Sr}/^{86}\text{Sr}$) agree with those normally assigned to precision Rb–Sr mineral/whole rock isochrons. The Rb–Sr age was calculated¹⁰, using an ^{87}Rb decay constant of $1.42 \times 10^{-11} \text{ yr}^{-1}$, with errors quoted at the 2σ level. A MSWD of 1.42 indicates that the data satisfy the criteria for an isochron.

The fluid inclusion Rb–Sr age $392 \pm 5 \text{ Myr}$ is in good agreement with the mean K–Ar age for the hydrothermal vein muscovites $387 \pm 6 \text{ Myr}$ determined by Rundle⁵. Furthermore the age of the main Lake District batholith, of which the Skiddaw Granite is part, has been established as $\approx 395 \text{ Myr}$ (Shap Granite, Rb–Sr age $394 \pm 3 \text{ Myr}$ (ref. 11), K–Ar age $397 \pm 7 \text{ Myr}$; Skiddaw Granite, K–Ar ages $392 \pm 4 \text{ Myr}$ (ref. 5) and $399 \pm 6 \text{ Myr}$ (ref. 12)). As the veins cannot be older than the granite and there is no reason to doubt the K–Ar vein muscovite age, the agreement verifies the fluid inclusion Rb–Sr age. A noticeable feature of the isochron (Fig. 1) is the large range in $^{87}\text{Rb}/^{86}\text{Sr}$ which minimizes the influence of the initial $^{87}\text{Sr}/^{86}\text{Sr}$ on the calculated age. This is attributed to a variable input of Rb throughout the period of quartz deposition, either from a purely magmatic source or released into the ore fluids as a result of concomitant wallrock alteration (that is, greisenization). High Rb values are consistent with the preferential partition of Rb into magmatic–hydrothermal solutions¹³. Because the inclusions are largely pseudosecondary in origin, no two samples will contain identical proportions of the same generation of inclusion. Hence there will be a natural variation in the observed $^{87}\text{Rb}/^{86}\text{Sr}$ ratios.

The fluid inclusion approach seems superior to techniques that rely on the complete resetting of pre-existing systems (that is, the annealing of fission tracks in wallrock minerals adjacent to mineral veins¹⁴). Furthermore being relatively stable, inclusions are less sensitive to subsequent thermal events which often overprint the true age of mineralization. This is especially so for K–Ar studies on wallrock clays which show a poor retentivity for radiogenic argon. Of comparable significance is the ease with which K–Ar systematics can be reset by later meteoric–hydrothermal activity¹⁵. Because later hydrothermal solutions will often take advantage of earlier mineralizing channelways, caution must be exercised when interpreting the K–Ar ages for feldspars and clays¹⁶. There is no *a priori* means of assessing whether a system has remained closed. It is also suspected that meteoric–hydrothermal activity may equally disturb Rb–Sr systematics. As yet the common Pb method for dating lead mineralization is only applicable to a small number of deposits of

the 'conformable' type. Recent refinements of terrestrial lead isotopic growth curves has done much to improve this technique and 'model' ages agree well with ages obtained by other methods. However, for most ore deposits, lead isotopic compositions indicate multistage histories which are not readily amenable to interpretation.

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Biogeochemical significance of a novel sedimentary C₂₇ stanol

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Recent studies^{1–3} of the lipids of marine organisms have revealed numerous novel sterols, many with modified side chains. As yet few of these compounds or their diagenetic products have been recognized in geological materials. However, recent investigations of various marine sediments^{4–8} have detected, for example, gorgosterol⁴ (IIo) and several 4-methylstanols^{7,8} (such as III/). Our gas chromatography (GC) and computerized gas chromatography–mass spectrometry (C-GC-MS) investigations of the extractable lipids in Neogene deep-sea sediments from the Japan Trench have revealed highly complex sterol distributions within which 69 different sterols have been characterized⁹. Several of these sterols^{9,10} (such as IIm, II n and III o), have been reported as constituents of marine organisms^{11–13}, but not of marine sediments; others are of unknown structure. Here we report the identification of one of these novel sterols, 27-nor-24-methyl-5 α -cholestan-3 β -ol (Ih) which supports the biological demethylation pathways proposed by Kobayashi and Mitsuhashi¹². Its diagenetic products would also include 27-nor-24-methylcholestanes which may be unrecognized components of the sterane mass fragmentograms widely used by petroleum geochemists for oil and source rock fingerprinting. Such components might also prove valuable marine markers in the interpretation of palaeoenvironments.

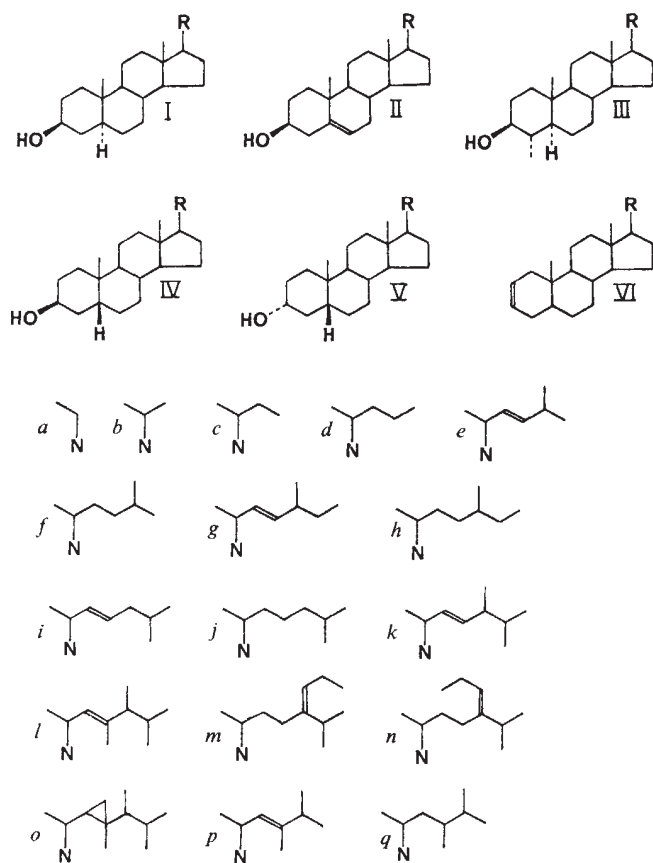


Fig. 1 Structural formulae of steroidal nuclei (N, I–VI) and side chains (R, a–q). The sterols I, II and III, bearing a wide range of side chains, are produced by organisms^{1–3,11–13,22–24} and occur in sediments^{4–10}. In contrast, the 5β-stanols (IV and V) and the Δ²-sterene (VI) are apparently derived from microbial and/or geochemical processes, and are often prominent components of immature sediments^{8,9,21,27,28}; however, the known variety of their side chains is less extensive than that of stenols and 5α-stanols.

During Legs 56 and 57 of the Deep Sea Drilling Project (DSDP), the Glomar Challenger drilled at seven sites in a transect of the Japan Trench^{14,15}. Our studies concentrated on the extractable lipids of 10 samples of Upper Pleistocene (~0.5 Myr) to Upper Miocene (~6 Myr) age, from four of these sites, with the aim of understanding the origin and diagenetic fate of the lipids^{9,10,16}. The analytical procedure used to obtain free sterol fractions from these sediments followed that previously described¹⁷, with subsequent modifications^{16,18}. Briefly, the extractable lipids obtained by sediment ultrasonication with solvent were divided into neutral and acidic components following saponification, and thin layer chromatography was used to separate the total neutrals further into fractions principally composed of hydrocarbon, ketone and alcohol constituents. Each fraction was analysed by capillary GC (SE-52 or OV-101 columns, deactivated by the barium carbonate technique of Grob *et al.*¹⁹) and C-GC-MS after derivatization (for example, of alcohols to their trimethylsilyl (TMS) ethers) when appropriate.

27-Nor-24-methyl-5α-cholestan-3β-ol (Ih) was identified from its GC retention time and mass spectral characteristics and by GC and C-GC-MS co-injection with a standard prepared by hydrogenation (PtO₂/H₂ in EtOAc) of ocellasterol (27-nor-24-methylcholest-5,22E-dien-3β-ol, IIg). Its mass spectrum (as a TMS ether) is indistinguishable from that of 5α-cholestan-3β-ol (Ij, Fig. 2), but the retention times of these two compounds differ (Fig. 3). Previous analyses of sedimentary sterols may not have

recognized 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) because of the prominence of 5α-cholestan-3β-ol (Ij) in such distributions and the necessity of fast scanning GC-MS (for example, 1 s scans) to distinguish the two compounds satisfactorily.

In the Japan Trench sediments, which are principally diatomaceous ooze and clays, the concentration of 27-nor-24-methyl-5α-cholestan-3β-ol (Ih), with the exception of one sample that contained only trace amounts (<0.1 ng per g dry sediment), varied from 0.6 to 11.7 ng per g dry sediment, values that correspond to 0.2 to 1.2 μg per g organic carbon. In four samples 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) was more abundant than 5α-cholestan-3β-ol (Ij), attesting its importance as a sedimentary sterol.

Although the origin of 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) is uncertain, it seems to be biological rather than geochemical. No apparent correlation exists between its abundance and that of the other 27-nor-24-methylcholesteroids with Δ^{5,22} (occellasterol (IIg)) and Δ²² (Ig) unsaturation, data that argue against its formation by geochemical reduction²¹. In addition, 27-nor-24-methylcholest-5-en-3β-ol (IIh) would be an expected product of such a nonspecific reduction process, but it was not detected in any of the sediments. Furthermore, the presence of only two 5β-stanols, 5β-cholestan-3β-ol (IVj) and 5β-cholestan-3α-ol (Vj), rather than a range commensurate with that of the Δ⁵-stenols and 5α-stanols indicates that reduction of Δ⁵-stenols is not a significant process in Japan Trench sediments and suggests that the 5α-stanols in the C₂₁–C₃₁ range⁹, are derived directly from biological inputs.

The identification of 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) means that both 27-nor-24-methylcholesteroids and 24-norcholesteroids are now known to occur as Δ^{5,22}-stenols and Δ²²- and 5α-stanols, supporting the demethylation pathways proposed by Kobayashi and Mitsuhashi¹². The organisms that act as the biological source of 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) are unclear. Its C₂₆ homologue, 24-nor-5α-cholestan-3β-ol (If) has only been isolated from a sponge²²; both this compound and other sponge constituents, such as C₂₁–C₂₄ 5α-stanols (Ia–d) (refs 23, 24), have been recognized in the Japan Trench sediments^{9,16}, where sponge spicules are also found^{25,26}. Hence, 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) may be of poriferan origin.

Considering the geological fate of 27-nor-24-methyl-5α-cholestan-3β-ol (Ih), at an early stage of diagenesis stanols can undergo dehydration, generating Δ²-sterenes^{27–29}. The Δ² C₂₇ sterene in the Japan Trench sediments which eluted just after cholest-2-ene (VIj) and possessed an identical mass spectrum is, therefore, probably 27-nor-24-methylcholest-2-ene (VIh); if so, then the diagenetic reactions of 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) parallel those of 5α-cholestan-3β-ol (Ij) (refs 8, 9, 27–29). Hence, 27-nor-24-methyl-5α-cholestan-3β-ol (Ih) could be a precursor for other sedimentary steroidal hydrocarbons with 27-nor-24-methyl side chains, particularly diasterenes³⁰, aromatic steroids³¹ and steranes, which might prove valuable indicators of marine sources of sedimentary organic matter. The possible existence of such 27-nor-24-methylcholestanes has a bearing on petroleum geochemical studies in view of the widespread use of steranes as biological markers³³. In particular, the process of diagenesis and maturation effect sterane isomerization, generating a wide range of stereoisomers^{32,33}; hence, mature sediments and oils would be expected to contain a comparable variety of 27-nor-24-methylcholestane isomers. Significant amounts of such compounds would markedly complicate the mass fragmentograms used for sterane evaluation^{32,33}, and also affect the quantitation of individual stereoisomers.

Novel compounds are likely to continue to be found in marine sediments because of the role of sediments as 'sinks' for organic matter. For example, the novel C₂₇ Δ^{5,22}-stenol, Δ²²-stanol and 5α-stanol in the Japan Trench sediments may possess 26,27-bisnor-23,24-dimethyl side chains (IIp, Ip and Iq in Fig. 3), as recently suggested³ for similar components in Walvis Bay diatomaceous ooze⁴.

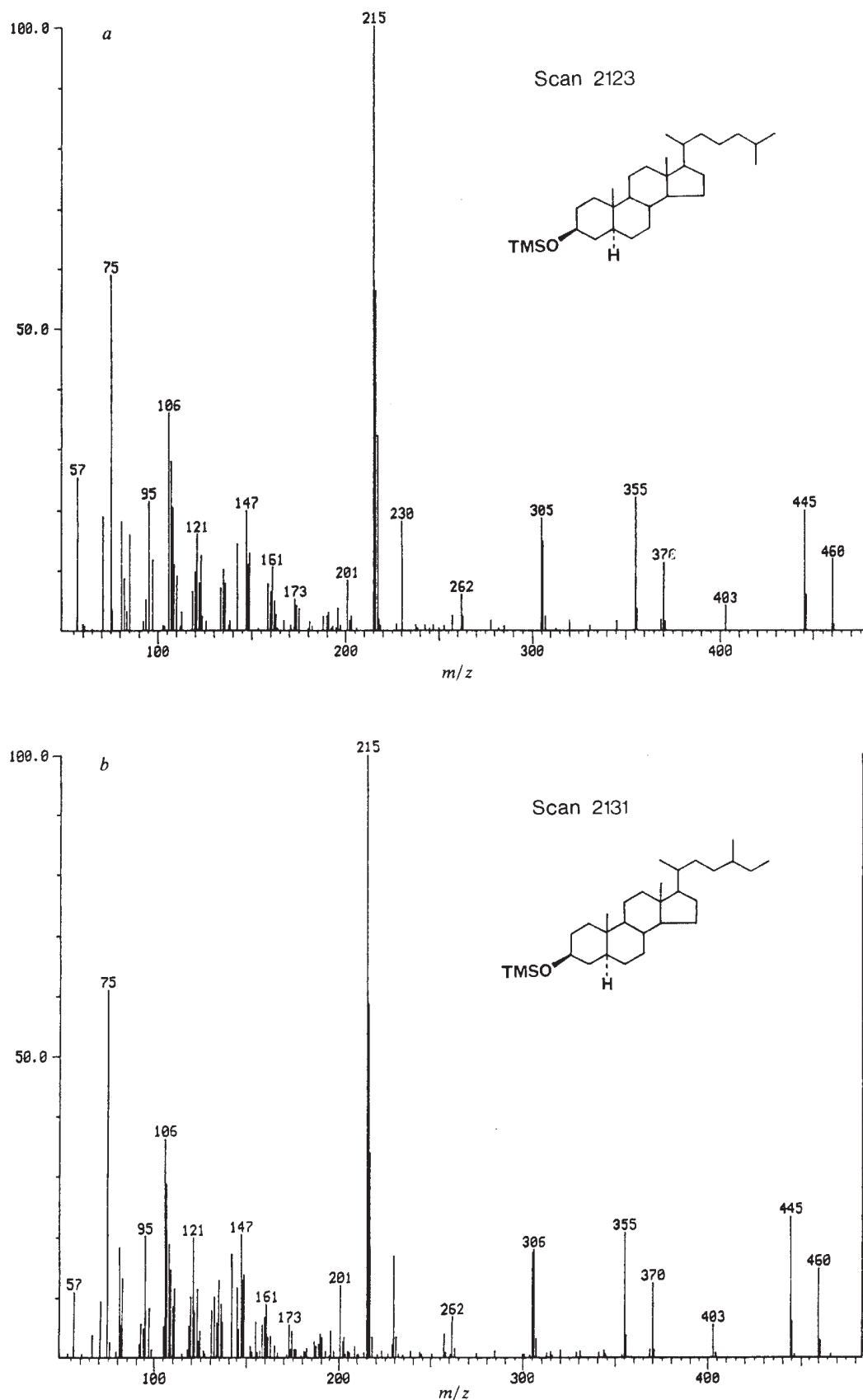


Fig. 2 EI mass spectra (40 eV, background subtracted) of: (a) 5α-cholestan-3β-ol (Ij); (b) 27-nor-24-methyl-5α-cholestan-3β-ol (Ih), as TMS ethers. The data were obtained from C-GC-MS analysis of the alcohol fraction separated from a Lower Pliocene diatomaceous claystone (DSDP Section 57-440B-39-3, 504m sub-bottom depth). The C-5 stereochemistry of (b) is assigned on the basis of its chromatographic characteristics and mass spectral fragmentation which are indicative of a 5α-stanol rather than a 5β-stanol⁸.

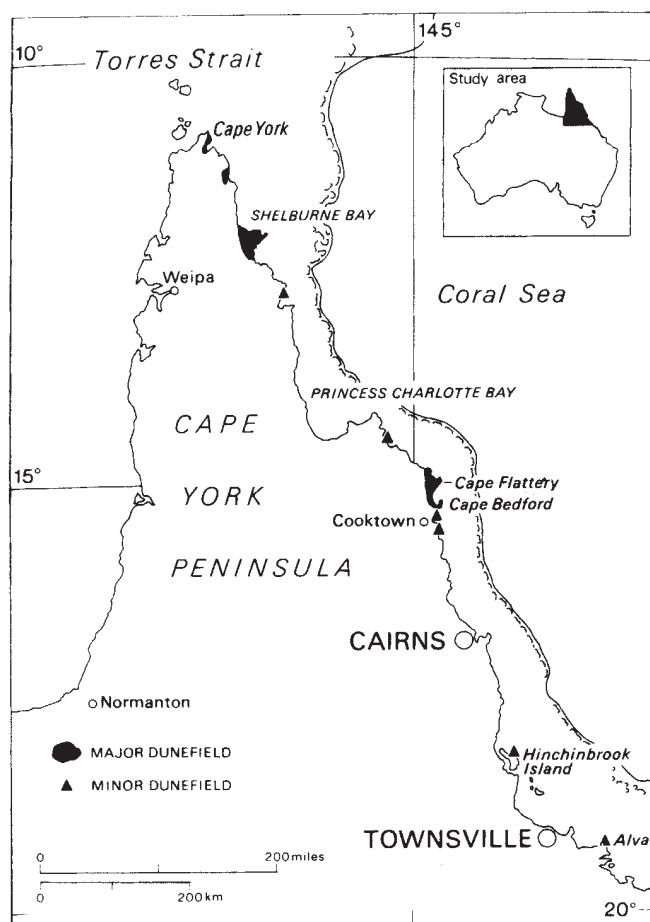


Fig. 1 Map showing location of Cape Flattery and other coastal dunefields in North-east Australia.

Some dunes show that part of the red pigment is being removed by progressive podsolization. A radiocarbon date obtained from a large root in growth position and embedded in humate⁹ exposed in coastal cliffs 3 km north-west of Cape Flattery indicated a 'background' age of $48,000 \pm 14 \text{ C yr BP}$ (Q-2085). Similar deposits occur in Southern Queensland¹⁰ and New South Wales where they have been shown to be last interglacial in age¹¹.

The forms of the second generation of dunes, which drilling has shown to overlie partly the first generation, are relatively sharp and the sands contain only very thin, non-indurated humate horizons. A typical weathering profile found in dunes of this generation is shown in Fig. 2. Two radiocarbon dates have been obtained from samples of charcoal collected at depths of 2 and 4 m respectively within this profile. The charcoal was pretreated with an alkali-pyrophosphate mixture followed by acidification to remove humic acid contaminants. The upper charcoal sample gave a date of $7,480 \pm 75 \text{ C yr BP}$ (Q-2081), and the lower sample indicated an age of $8,200 \pm 85 \text{ C yr BP}$ (Q-2082). As the charcoal fragments must have been incorporated within the sands while the dune was active, the dates suggest that stabilization and weathering has taken place within the past 7,500 C yr . The possibility that the charcoal is reworked from older deposits cannot be entirely ruled out, and in this event the dates would indicate a maximum age for the reddened dune.

The youngest dune sand unit at Cape Flattery includes all the active and recently active dunes which have partly buried and reworked the older sands. These dunes mostly consist of undifferentiated white quartz sand, but those which have been stabilized longest show immature podsol development with brown or brownish-orange B horizons. Buried organic Al

horizons are exposed in the walls of some dunes. Two radiocarbon dates on *in situ* wood stumps from one such buried soil including a 1 m-thick brownish B horizon both indicated 'modern' ages (SRR-1534 and SRR-1535).

Previous work on red sediments has led to the conclusion that reddening is generally slow, and in arid regions may take 10^5 – 10^6 yr (refs 12, 13). However, the evidence reported here suggests that in humid tropical conditions bright red colours may be attained within 10^3 – 10^4 yr. Furthermore, over a longer time period much of the initial red colour may be lost as humic and fulvic acids accumulate within the profile. A similar conclusion was reached, though without radiocarbon evidence, by Andrieuse¹⁴ in relation to reddened beach ridges and alluvial deposits in Sarawak.

Rapid reddening of stabilized dunes at Cape Flattery is favoured by several factors. First, abundant detrital ilmenite and magnetite grains within the sands provide a large potential source of iron which can be released by weathering^{15–19}. Second, rapid release of iron and formation of ferric oxides is favoured by oxidizing interstitial conditions which reflect the good drainage of the dune sands and the seasonally humid tropical climate. At Cooktown, located just south of Cape Flattery, mean daily temperature is above 25°C throughout the year, reaching 31.3°C in December, while about 75% of the mean annual rainfall occurs between January and April. Third, weathering of ilmenite, magnetite and other minerals may be enhanced by organic acids released by the heath and woodland vegetation characteristic of the dune areas^{20,21}. Soil pH values at Cape Flattery generally lie in the range 3.5–5.5.

Rate of reddening in dune sands and other recent sediments is dependent on several variables including sand mineralogy, temperature, moisture availability, drainage conditions, degree of sediment stability and significance of airborne dust input²². Climatic conditions undoubtedly permit reddening in both the arid and humid tropics, but, other things being equal, the time required to attain a given degree of redness is likely to be longer in the arid tropics due to lower moisture availability, a slower rate of mineral weathering and relative unimportance of biochemical processes. In humid tropical areas periods of seasonal

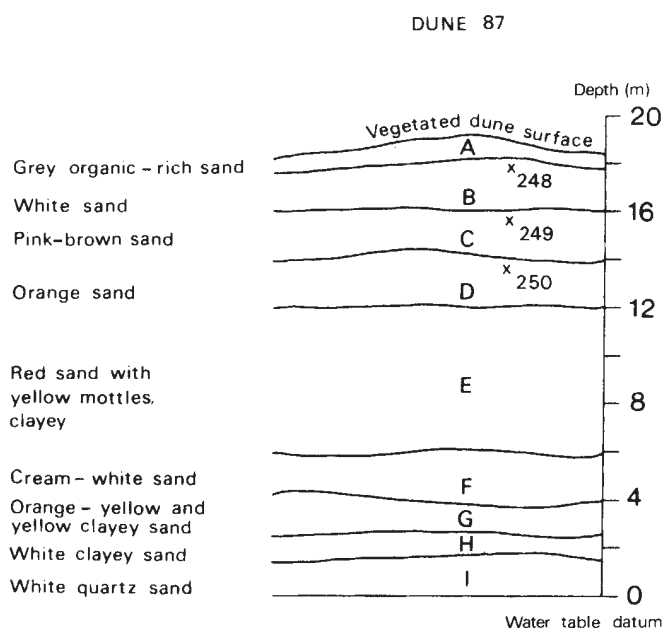


Fig. 2 Diagrammatic representation of a weathering profile exposed in a section through a stabilized dune near Cape Flattery. Radiocarbon dates Q-2081 and Q-2082 refer to charcoal samples (x) 248 and 249 respectively. Charcoal sample 250 was analysed for chemical purposes.

drought and water table lowering can also be expected to favour the formation of haematite²³. However, owing to the diverse nature of the controls on reddening, time cannot be regarded as an independent variable in the reddening process, and colour alone cannot be used as an adequate guide to age or morpho-stratigraphic correlation between different areas.

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Internal plumbing of Mount Etna

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A number of conflicting models have been proposed for the internal plumbing system of Etna; some of the conflict arises from attempts to fit the same model to the whole of Etna's history. We argue that at present magma reaches the surface without significant high-level storage during its ascent, and that eruptions are tectonically controlled. These conditions have probably prevailed for the past 200 yr. However, evidence from lavas erupted in prehistoric times suggests that substantial low-pressure differentiation accompanied by different styles of eruption took place, implying a different plumbing system which includes high-level storage of magma.

Etna is a central-vent volcano some 3 km high, with numerous parasitic cones. The position of the central conduit has shifted with time, each change in location generally being preceded by caldera collapse¹. Almost continuous activity occurs at or near the summit where there is a steady effusion of lava, normally at the rate of less than $1 \text{ m}^3 \text{ s}^{-1}$, accompanied by strombolian activity. A curious aspect of summit activity is that two craters less than a few hundred metres apart may simultaneously show quite different activity; for example, in 1966 to 1971 the north-

east crater was erupting lava on the surface, while only 200 m away, activity in the chasm was $\sim 1,000 \text{ m}$ below this level. Persistent summit activity usually stops when flank eruptions occur—these are characterized by much higher rates of effusion, typically at tens of cubic metres per second. Feeding fissures tend to parallel the complex regional tectonic pattern.

Based on petrological evidence, Rittmann² envisaged that magma is supplied under Etna by abyssal fissures and that there is no magma chamber. Guest³ suggested the existence of a plexus of dykes and sills within or just below the volcanic pile, analogous to that proposed for Kilauea⁴. From horizontal ground deformation studies between 1971 and 1974, Wadge^{5,6} postulated two magma reservoirs: one at the base of the volcanic pile, and the other an open cylindrical reservoir in the upper part of the central conduit. The central conduit reservoir, known as the chasm, was envisaged by Wadge as feeding flank eruptions by first opening fissures radial to the chasm as a result of hydraulic fracturing and subsequent drainage of the reservoir under gravity through these fissures. Tanguy and Kieffer⁷ suggested high-level magma storage in active dyke-like intrusions, eruption from which was triggered by tectonic activity.

Studies of vertical ground deformation have been made between 1975 and 1980⁸. During this time persistent activity occurred from the north-east crater and the north-east rift between 1975 and 1978, and flank eruptions occurred on the southern flank between May 1978 and August 1979. Following the established deformation pattern at Kilauea, while relatively little deformation might have been expected from some of the persistent activity at the summit, substantial effusion low on the volcano flanks should have been preceded by inflation caused by high-level magma storage, followed by deflation as the chamber emptied. However, during 1975–80, including the major August 1979 flank eruption during which an estimated minimum of $8 \times 10^6 \text{ m}^3$ of magma was erupted (C. Kilburn, personal communication), no gross pattern of inflation or deflation was observed either by precise levelling traverse across the summit, or from some 35 dry-tilt stations. Based on five years of tilt measurement on Etna, there is no evidence that large amounts of magma are stored within the volcano before eruption, and if it is stored in an intermediary reservoir, this lies well below the base of the volcano. Thus internal conditions at Etna seem to differ from those of Kilauea.

Although no gross accumulation of magma within the Etna volcano can be identified, small vertical movements do occur. A 1.5-cm inflation area of $\sim 1 \text{ km}$ radius was observed two years before the May 1978 eruption in the region of the main vent, suggesting that a narrow wedge of magma was emplaced below the eruption site before effusion started⁸.

Although the vertical deformation results do not support a high-level magma chamber in a conventional sense, they are not inconsistent with Wadge's⁶ proposal of an open chamber in the chasm at the top of the central conduit. However, Wadge's model requires that the chasm drains during the course of flank activity and that the amount of lava drained corresponds to the volume of lava erupted. These conditions have not been fulfilled by recent eruptions when, for example in April 1971, the chasm floor was lower than the active flank vents³. Thus, although the Wadge model is not discounted as a potential mechanism, it cannot be considered as a general model to explain Etna's plumbing system during 1970–80 (see also ref. 9).

Based on seismic evidence, Sharp *et al.*¹⁰ suggested a magma storage region at a depth of $\sim 20 \text{ km}$ below Etna. This has the form of a prolate ellipsoid with a width $\sim 20\text{--}30 \text{ km}$ and the long axis paralleling the north-east fissure trend through the mountain. From the same experiment they identified the Moho at 27 km, magma generation occurring below this level.

During historical times the lavas have been basic hawaiites with little variation in chemical composition^{11–13}. Historic lavas are strongly porphyritic with 30–40% phenocrysts of plagioclase, augite and olivine, plagioclase being the dominant phenocryst phase; the magma therefore undergoes extensive

crystallization before eruption. Limited variation in chemistry between lavas indicates that there is little differentiation. The predominance of plagioclase suggests that most of the crystallization takes place at moderate to low pressures. Experimental work¹⁴ on an alkali basalt shows that crystallization of plagioclase is suppressed to near solidus temperatures at pressures approaching 9 kbar, equivalent to ~22 km depth. These observations imply that considerable crystallization is taking place at relatively high levels, but the magma is erupted before significant differentiation occurs. This is consistent with the ground deformation interpretation that there is no substantial high-level magma storage. Magma stored in the open central conduit may, however, undergo vapour-phase fractionation¹⁵⁻¹⁸.

Although the composition of historical lavas has remained almost constant, there are petrographic distinctions, for example the 1974 flank eruption lavas^{7,19,20}, where plagioclase was not a primary phenocryst phase. In this case either the crystallization of plagioclase was suppressed by high P_{H_2O} conditions, or magma migrated from depth rapidly containing only early formed phenocrysts of augite and olivine.

The evidence given above supports the view expressed by Rittmann² that on Etna magmas ascend directly to the surface

from depth without storage in a high-level magma chamber. These observations agree to some extent with the model proposed by Tanguy and Kieffer⁷ of a system of active intrusions. It is likely that magma enters the volcanic pile permissively and a high level of magma in an open central conduit gives steady persistent activity. Dyke-like intrusions leading to flank eruption radiate at various depths from the central conduit and may be emplaced in tectonically dilated fissures. Eruptions are controlled both in space and time by the interaction of magma fluid pressure and the regional stress pattern (see ref. 21): a decrease in the minimum horizontal principal stress will increase the gape of dilational fissures, allowing an increased rate of magma uprise. The presence or absence of a cinder cone over the vent, for flank eruptions, will depend on the depth of connection between the feeder dyke and the conduit (Fig. 1a). Eccentric cones result from ascent of magma independent of the central conduit, for example, the Moio cone 18 km north of the summit²². The role of the 20-km deep magma reservoir is uncertain, but the observation that repose time between the end of a flank eruption and the onset of persistent activity is proportional to the volume of erupted material in the flank eruption⁶ may suggest that the repose period is controlled by the time taken for the reservoir to be replenished.

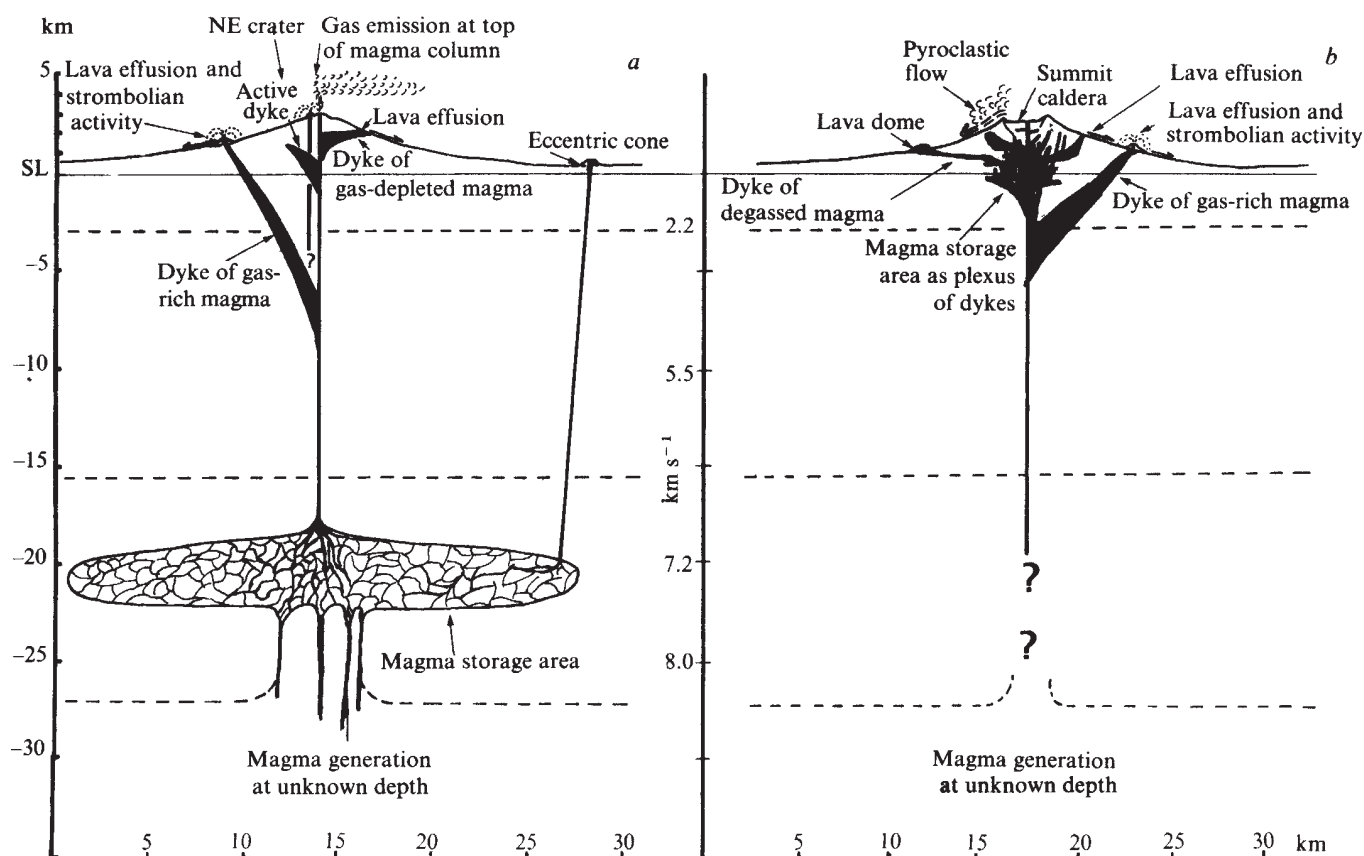


Fig. 1 *a*, Interpretation of the present internal structure of Mount Etna. Magma rises up the central conduit from a deep magma storage area. Low effusion rate persistent activity from this conduit occurs near the volcano summit. A column of magma is probably always present in the central conduit and associated conduit of the north-east crater, at levels of less than a kilometre below the volcano's summit. The opening of fissures by tectonic activity allows magma to leave the central conduit at different depths to produce flank eruptions with high effusion rates compared to persistent activity. Occasionally eccentric eruptions may occur as a result of magma rising directly from the deep magma storage area using a conduit not related to the central conduit. The dimensions of the magma storage area and the seismic velocity profile are from ref. 10. *b*, Condition of the volcano during pre-Valle del Bove times, showing high level plexus of dykes in which magma is stored. Although the different styles of activity are shown on the same diagram, the normal activity was probably strombolian with occasional extrusions of volcanic domes and paroxysmal events.

Geophysical information is only available for the past few years on Etna. Nevertheless, interpretations of internal plumbing based on these data are probably relevant for the past 200 yr, during which time the petrology of the lavas, styles of eruption and output of the volcano have been relatively constant. However, before 1669 the output of the volcano was considerably higher²² and the erupted lavas petrographically different from the more recent ones, although of hawaiitic composition. These earlier lavas are characterized by larger phenocrysts of plagioclase, typically 4 mm long, compared with those in later lavas which are ~1 mm long. The presence of fewer phenocrysts may imply a short period of high-level storage. A plexus of active dykes near the base of the volcanic pile may have existed at this time⁶. Thus, even during historic times the internal plumbing of Etna seems to have changed; these alterations may have resulted from variations in the regional stress pattern.

At times in the prehistoric past of Etna (5,000–100,000 yr BP), before formation of the Valle del Bove¹, a very different system of internal plumbing may have operated in the volcano. Among the older volcanics, evolved lavas of mugearitic and benmoreitic composition are relatively common, indicating more extensive differentiation of the ascending magma^{11–13}. Plagioclase is considered to have played a major part in this crystal fractionation¹⁸ suggesting that at one time there was high-level magma storage. Ash fall and ignimbrite deposits occur^{23,24}, indicating violent explosive events in the summit region, possibly associated with caldera collapse and de-gassing of a high-level magma reservoir (Fig. 1b). On the lower flanks of the volcano, relatively degassed magma was erupted, which formed benmoreitic autoclastic domes¹⁸ probably fed from a storage area that had been degassed by loss of volatiles up the central conduit.

We make the following conclusions: (1) there is no simple model of volcano plumbing that can be applied to central basaltic volcanoes, and a long-lived central volcano made up of repeated eruptions may not have the same type of plumbing throughout its history. (2) Petrogenetic models based on a whole suite of lavas spanning a long time period may be misleading as in this case, where different models involving the presence or absence of a high-level magma reservoir are applicable to different phases in the volcano's plumbing history. (3) Because a volcano's plumbing is controlled by factors such as the regional stress pattern, rate of magma supply and rate of stress adjustment in the magma source region, a study of Etna's changing internal conditions may have direct relevance to the regional tectonic evolution.

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Anti-predator function of bluegill sunfish nesting colonies

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Although the adaptive significance of nesting in colonies has been investigated repeatedly in avian species, until now no detailed studies have been reported on colonial nesting in fishes. Investigators of avian colonial systems have suggested that birds nest in colonies either as a response to predators^{1,2} or to locate more easily ephemeral food resources^{3,4}. The avian literature is unclear, however, as to which selective pressure (predation or food resources) is more crucial, with investigators using similar evidence to reach essentially opposite conclusions⁵. I report here evidence obtained from a long-term study of a colonial fish, the bluegill sunfish (*Lepomis macrochirus*), showing extreme synchrony in breeding and the additional protection afforded offspring by tightly clustered groups of adults, which argues strongly that colonial nesting in this species and other fishes has evolved in response to predation pressure.

Observations on the reproductive system of the bluegill sunfish were made during the summers of 1976, 1978 and 1979 while skin or scuba diving in Lake Cazenovia, New York. In this lake, male bluegills construct nests (bowl-shaped depressions in the substrate) in colonies ranging in size from 15 to 500 nests. Throughout the breeding season males periodically reoccupy, breed in, and abandon these nesting areas. Although suitable nest sites are not limited (in preparation), during the spawning period occupied nests are packed rim to rim, with most nests having five to seven contiguous neighbours (Fig. 1).

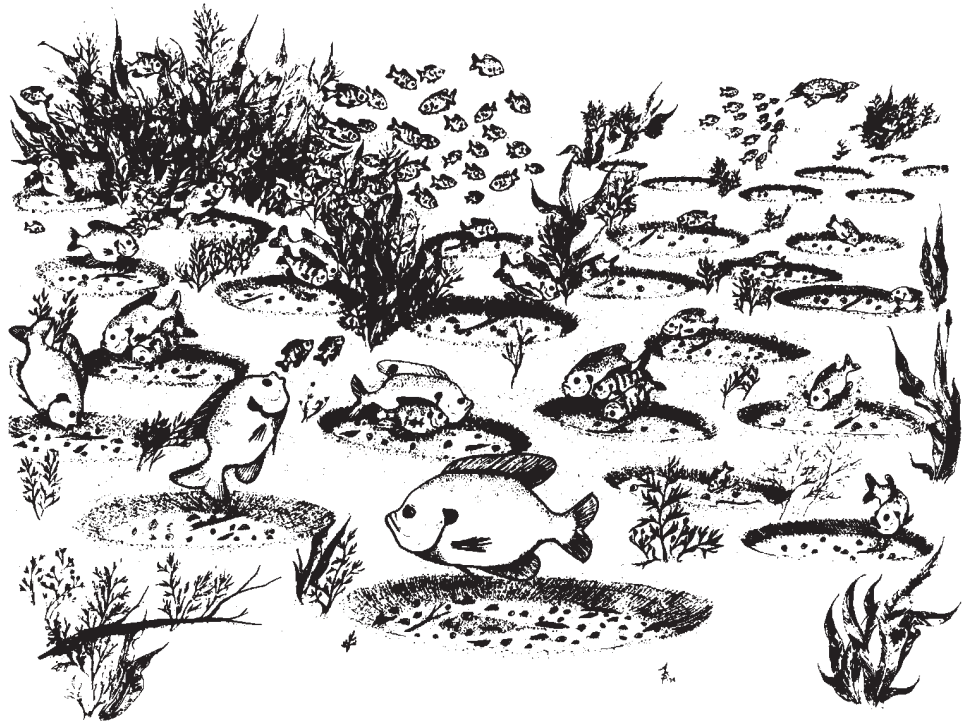
As early as 1938, Darling⁶ suggested that colonially nesting birds might breed synchronously to reduce the time their young are available to local predators. However, in avian systems, an alternative hypothesis also predicts breeding synchrony. If nesting colonies serve as 'information centres' that enable unsuccessful or inexperienced foragers to follow more successful individuals to ephemeral food sources, individuals breeding in synchrony would gain an advantage⁷. Because colonially nesting fishes typically neither feed their young nor leave the colony to forage, the competing hypothesis of social foraging does not apply. Consequently, the presence of breeding synchrony in these organisms would provide stronger support for the predation hypothesis than synchronous breeding in avian systems.

If predation is the evolutionary force favouring colonial nesting in bluegills, individuals within a colony should synchronize their reproductive efforts, resulting in greater breeding synchrony within colonies than between colonies. As predicted by this hypothesis, breeding within bluegill colonies in Lake Cazenovia is remarkably synchronous. At a particular colony breeding episodes occur periodically, and consist of the establishment of males on nests, the arrival of gravid females which remain in the water column until spawning is initiated, and a brief 1- or 2-day spawning period. Between these spawning periods gravid females are absent and successful males provide parental care. Although common responsiveness to optimal breeding conditions seems to promote a degree of breeding synchrony among all the colonies in Lake Cazenovia, synchrony within colonies is much greater than between colonies, as predicted, and even neighbouring colonies can cycle out of phase.

The period immediately following the termination of parental care is often critical to an individual's survival. During this period, bluegill fry minimize their availability to predators by abandoning their nests synchronously, with most of the offspring in an entire colony leaving within a period of a few hours. Analogous fledgling synchrony has been reported in some

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Fig. 1 An artist's conception of the activity in a bluegill colony during the spawning period. An aggregation of females is shown above a spawning centre (top centre). Multiple spawning partners (centre right) can indicate the presence of a 'female mimic'¹², a small sexually mature male that mimics female behaviour while 'stealing' fertilizations from nesting males. Nesting males are shown mobbing a large predatory turtle (top right), fanning eggs (right middle) and swimming rapidly around the perimeter of their nests in 'rim circling' displays (bottom right).



colonial birds⁸. In fish, as in birds, the impact of predation by locally available predators should be reduced when large numbers of offspring become available over a short period of time (as many as 100,000 in a single bluegill nest). The extreme synchrony observed both in breeding and nest abandonment argues strongly that colonial nesting in bluegills serves an anti-predator function.

In addition to synchronous breeding, active defence can limit the availability of the offspring of colonially nesting animals to predators. In a colony where each parent defends an area around its nest, if defended areas overlap, the offspring might benefit from the cumulative defence of several individuals. The offspring of colonially nesting birds have been found to gain such an advantage, with nests in high-density areas being better protected than those in low-density areas⁵, and this type of advantage has also been suggested for colonially nesting fishes⁹⁻¹¹. In the case of bluegills, evidence of a colony structure that promotes overlap of defended areas would support further the hypothesis that colonial nesting is a response to predation pressure, as would evidence that offspring in high-density areas gain additional protection.

Female bluegills select mates such that males guarding nests are found in more tightly packed groups than one would predict if females were indiscriminately selecting mates. During the spawning period, gravid females are attracted to spawning pairs and deposit their eggs in adjacent nests, creating areas of spawning activity or 'spawning centres'^{12,13}. Bluegill males whose nests are not located in these spawning centres often fail to receive eggs, or receive too few eggs to merit parental care, and abandon their nests following the spawning period. Might the spawning response of female bluegills function to ensure that their offspring are adequately protected from predation by a tightly compacted group of defending males?

Attempted predation was common in the bluegill colonies of Lake Cazenovia, occurring more frequently than has been reported in avian colonial systems. In a typical 10-min observation period, a bluegill male defended its nest several times against potential egg predators. In 950 min of cumulative observation, the most common piscine egg predators were foraging (not nesting) conspecifics (72%) and members of the closely related species *Lepomis gibbosus*, the pumpkinseed sunfish (21%).

To test whether the offspring in nests in tightly clustered groups receive additional protection against predators, I

removed males from both isolated and surrounded nests, then recorded the number of egg predators present in the nests at 1-min intervals for 10 min. Nesting males were not observed to forage in temporarily vacated nests. Isolated nests (having no more than one neighbouring nest) were more vulnerable to egg predators than were surrounded nests (having five or more neighbouring nests) (Fig. 2). Not only did the presence of defending neighbours make it more difficult for foraging fishes to enter a focal nest, but neighbouring males often actively attacked egg predators foraging in the focal parentless nests, with isolated nests receiving significantly fewer defences by neighbours than surrounded nests (Fig. 2). The additional protection afforded to nests in high-density areas is expected to result both from the 'selfish herd' effect¹⁴, by which individuals passively gain from the presence of neighbours, and from the overlap of defended areas. Both protective effects should extend readily to other types of predators attacking bluegill offspring.

The biological significance of these findings is supported by two observations. (1) Offspring in parentless nests sometimes survive to the freeswimming stage because of the incidental protection of neighbouring males. (2) Foraging schools composed of bluegills and pumpkinseeds sometimes harass isolated nesting males until these males are no longer effective at nest defence; this results in complete loss of brood. I never observed the nests of males in high-density areas to be similarly overrun, apparently because of the incidental protection provided by neighbouring nest-defending males. The inability of isolated males to defend their nests against egg predators, especially those foraging in schools, has probably been a major selective force promoting colonial nesting in centrarchid fishes.

This hypothesis is supported by the observation that those centrarchids which frequently nest solitarily or in loose association (for example, *Micropterus*, *Ambloplites* and *Pomoxis*) are larger, more piscivorous fishes than those that typically nest colonially, such as *Lepomis macrochirus*, *Lepomis megalotis* or *Lepomis punctatus*, even in the same habitat. These larger, more piscivorous forms are probably able to defend their nests better against foraging schools¹⁵. Such superior capability alone, however, is an inadequate defence for isolated nests when sunfish are present in high densities. In these conditions the nesting success of even the largest, most piscivorous forms, such as *Micropterus*, can be severely limited¹⁶. Finally, as foraging schools of bluegills are such predominant and effective predators on the eggs of conspecifics, wherever bluegills occur and

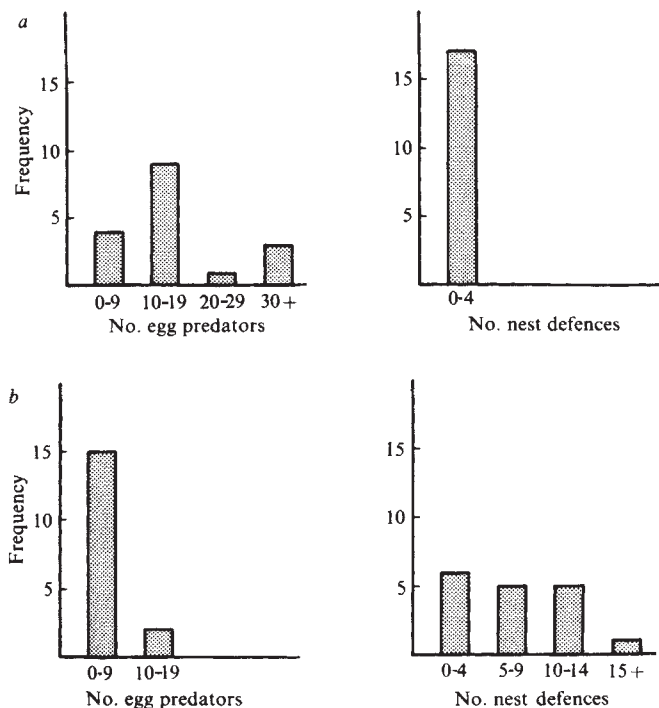


Fig. 2 Two observers simultaneously removed nest-guarding males from *a*, isolated nests (having no more than one neighbouring nest) and *b*, surrounded nests (having five or more neighbouring nests), then recorded the number of egg predators present in each nest at 1-min intervals for 10 min, and the total number of defences by neighbouring males. Surrounded nests had significantly fewer cumulative egg predators ($P < 0.001$, Mann-Whitney) and received significantly more defences by neighbouring males ($P < 0.001$, Mann-Whitney).

nest, an effective egg predator will also occur. Thus bluegills might be considered their own worst enemies, and may themselves have generated much of the selective pressure promoting their extreme form of colonial nesting.

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Induction of subacute murine measles encephalitis by monoclonal antibody to virus haemagglutinin

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Although the events which predispose a host to measles virus persistence remain largely unknown, measles antibody has been shown to contribute to the production of a persistent infection by this virus both *in vivo* and *in vitro*. Thus, the addition of measles antibody to cells infected with measles virus promotes virus persistence^{1,2}. Latent measles infection occurs in newborn hamsters with maternally acquired antibody after inoculation with measles virus in the immediate neonatal period³. A subacute encephalitis with measles virus persistence has been induced in weanling BALB/c mice that received antibody after virus inoculation⁴ and in measles-immune primates infected with a virus derived from a patient with subacute sclerosing panencephalitis⁵. Measles virus consists of six polypeptides, and treatment of measles-infected cells with antibody has been shown⁶ to alter the pattern of their synthesis. As the antigenic specificity of the antibody responsible for these observations is not known, we decided to investigate the effects of monoclonal antibodies directed against the individual measles polypeptides. We report here that a monoclonal antibody directed against the virus haemagglutinin, unlike an antibody to the virus nucleocapsid protein, is able to induce a subacute encephalitis *in vivo*.

After the intracerebral (IC) inoculation of weanling BALB/c mice with $10^{2.7}$ ICD₅₀ units of hamster neurotropic (HNT) measles, about 70-80% of the mice die from an acute encephalopathy. This encephalopathy produces mortality within the first 3 weeks and is characterized by a complete absence of brain inflammation, although neuronal viral antigen is readily demonstrable⁷. If hyperimmune measles antiserum (HI serum) is administered 3 days after virus inoculation, all the mice survive the acute period, but ~30% develop a delayed disease and die. Its onset is variable and has been observed up to 8 weeks post-inoculation. It is characterized by weight loss, seizures, and multiple neurological abnormalities that are fatal in a few weeks. Unlike the acute encephalopathy where brain inflammation is absent, histopathology of this subacute disease is characterized by mild inflammatory response of the meninges and parenchyma. Thus, it is referred to as an encephalitis. Immunofluorescence of the frozen sections from moribund animals shows abundant viral antigen in neurones of the hippocampus, frontal cortex, brainstem and cerebellum⁴. Mice which do not develop the subacute disease (~70%) have been observed for up to 6 months and remain well.

The HI serum used to induce the subacute encephalitis was prepared by hyperimmunization of BALB/c mice with Edmonston measles virus⁴. The serum has reactivity to all polypeptides of the measles virion. However, the polypeptide specificities of the antibody molecules in this panreactive serum that are responsible for this *in vivo* effect are unknown. To investigate this question, ascitic fluid containing monoclonal antibodies with single specificities against the virus haemagglutinin (C1 AF)⁸ and virus nucleocapsid protein⁹ was tested in this system for the ability to modify disease. Administration of the C1 AF abolished the acute encephalopathy and led to the development of a subacute disease which lasted up to 70 days. Two representative experiments, out of eight, are shown in Table 1. Large doses of C1 AF inhibited disease completely and with decreasing amounts of antibody the incidence of the

Table 1 Mortalities of BALB/c mice after intracerebral inoculation of HNT measles virus—effects of passive transfer of monoclonal antibody to viral haemagglutinin

Expt no.	Group	Antibody	Dilution	Mortality		Mean survival time (days)	Daily mortalities
				Acute	Delayed		
1	A	None	—	70 (7/10)	0	11 (8–19)	d8 ³ , d9 ² , d16 ¹ , d19 ¹
	B	HI serum	1:10	0	50 (8/16)	39 (24–56)	d24 ¹ , d27 ¹ , d38 ³ , d41 ¹ , d48 ¹ , d56 ¹
	C	P3 X63 AF	1:10	70 (7/10)	0	14 (12–18)	d12 ² , d13 ³ , d17 ¹ , d18 ¹
	D	79 X1 C1 AF	1:25	0	30 (3/10)	56 (26–71)	d26 ¹ , d70 ¹ , d71 ¹
			1:100	0	40 (4/10)	43 (35–56)	d35 ¹ , d38 ¹ , d42 ¹ , d56 ¹
2	A	None	—	72 (8/11)	0	12 (9–19)	d9 ¹ , d10 ² , d11 ² , d12 ¹ , d14 ¹ , d19 ¹
	B	79 X1 C1 AF	1:10	0	0	—	—
			1:20	0	20 (2/10)	46 (36–56)	d36 ¹ , d56 ¹
			1:100	0	36 (9/25)	37 (28–45)	d28 ² , d32 ² , d41 ³ , d43 ¹ , d45 ¹
			1:500	10 (1/10)	40 (4/10)	25 (20–34)	d20 ¹ , d22 ² , d25 ¹ , d34 ¹

Four-week-old female BALB/c mice were inoculated with $10^{2.7}$ ICD₅₀ units of HNT measles virus in 0.03 ml in the right cerebral hemisphere under light ether anaesthesia, using a tuberculin syringe and 30 G needle. All animals received 0.2 ml of the appropriate dilution of antiserum through the tail vein on day 3 post-inoculation. Control animals received nothing. Transfer of normal mouse serum in previous experiments were without effect on the onset or course of clinical disease. Characteristics of antisera used were: HI serum, serum pooled from 50 female BALB/c mice after hyperimmunization with purified Edmonston measles virus. Initial immunization was with complete Freund's adjuvant followed by multiple boosts (13 boosts over 18 months) in saline or incomplete Freund's adjuvant. The serum titres were: 1:1,024 by haemagglutinin inhibition test, 1:2,048 by complement fixation test, $>10^{-4}$ by virus neutralization test and 10^{-6} dilution by radioimmunoassay. The serum reacts with all polypeptides of the Edmonston measles virus in a radioimmunoprecipitation assay³. PS X63 AF, ascitic fluid prepared from pristane-primed BALB/c mice inoculated with the parent myeloma cells used to produce the 79 XI-C hybridomas⁶. 79 XI C1 AF, ascitic fluid prepared from pristane-primed BALB/c mice inoculated with 79 XI-C hybridoma. The ascitic fluid had reactivity only with the haemagglutinin of Edmonston measles virus. The ascitic fluid titres were: 1:4,000 dilution in virus neutralization, 1:4,000 in haemagglutinin inhibition and 10^{-7} dilution in a solid-phase radioimmunoassay. The antihaemagglutinin antibody is identified as IgG1 subclass¹. All mortalities were recorded from the day of virus inoculation. Mortalities within the first 21 days were arbitrarily considered 'acute' and subsequent mortalities were included in the 'delayed' group. Mean survival time was calculated as the mean duration from the day of inoculation to the day of death (range indicated in parentheses). Daily mortalities; superscript number indicates the total mortality for the day (d) indicated.

subacute clinical disease increased. At the greatest dilution (1:500) used, protection from the acute disease was not absolute (expt 2, Table 1). In animals receiving the lower doses of antibody the onset of the subacute illness was earlier and the total mortality greater. These observations suggested an inverse correlation between the dose of antibody transferred and total mortality, and, furthermore, that the onset of clinical illness may be related to the fall in serum antibody. This was supported by studies correlating the antibody levels with the onset of clinical illness. Following transfer, the antibody levels declined, and were barely detectable when clinical illness began. Transfer of large amounts of monoclonal antibody to the virus nucleocapsid protein did not affect the acute mortalities in three experiments. Similarly, no effect was observed with normal mouse serum or ascitic fluid obtained from the parent myeloma (P3 X63)⁸.

The virological and pathological features of the delayed encephalitis were studied. One hundred weanling BALB/c mice were inoculated with $10^{2.7}$ ICD₅₀ units of HNT measles virus and given 0.2 ml of a 1:100 dilution of the C1 AF through the tail vein on day 3. Every 5 days viral expression in the brains of five infected animals was studied by direct immunofluorescence. A fluorescein-conjugated IgG purified from the serum of a patient with subacute sclerosing panencephalitis capable of reacting with all measles polypeptides was used¹⁰. On day 8 after virus inoculation, the brains of all five animals had viral antigen, which was detectable in occasional neurones. On days 13 and 18 viral antigen could no longer be detected in any of the 10 brains studied. At day 23 viral fluorescence was readily observed bilaterally in all five animals. The onset of clinical illness followed shortly afterwards in the remaining mice. The animals with clinical encephalitis had viral antigen detectable in the neurones of the hippocampus, frontal cortex, brain stem and cerebellum. Brains from moribund animals were also studied by light microscopy. These showed meningeal and parenchymal perivascular cuffs of mononuclear cells. However, the degree of inflammation was considerably less than in chronic encephalitis described in the SJL mouse¹⁰ or disease induced by the panreactive serum in BALB/c mice⁴.

Clearly, unlike the antibody to nucleocapsid protein, antibody with reactivity only to the virus haemagglutinin can modify the clinical course of acute murine measles encephalitis. Although

the precise mechanisms whereby C1 AF delays the acute encephalopathy are unknown, the effect may be through antigenic modulation *in vivo*. The haemagglutinin is expressed on infected cell surfaces and is available for modulation by antibody. The observed disappearance of all viral antigens from the central nervous system at days 13 and 18 suggests that the reaction between the haemagglutinin peptide and the antihaemagglutinin antibody alters the synthesis of all viral polypeptides *in vivo*. Similar effects have been observed in experiments *in vitro*⁶.

The HI serum contains antibody against the fusion protein which is also expressed on infected cell surfaces. Although antibodies to fusion protein may also be important in antigenic modulation of infected cells, *in vitro* studies suggest that this antigen is available for interaction with its antibody only in the Fab monomeric state at least on the surface of some infected cells¹¹. However, monospecific antibody to the fusion protein of another paramyxovirus, SV5, inhibited the spread of infection by limiting cell fusion and virus entry after virus adsorption¹². The effect of antibody to the fusion protein on the modulating ability of the HI serum remains to be determined. The results from this study establish that antibody to one surface component, the haemagglutinin, is responsible for some of the *in vivo* effects of the HI serum. The antibody reactivity not only leads to delayed clinical disease but alters the synthesis of viral polypeptides in infected cells.

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Acute rabies death mediated by antibody

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Among inadequately immunized laboratory animals dying of rabies, a small but consistent proportion succumb after an incubation period shorter than that of any of the unvaccinated controls^{1-3,7} and this phenomenon has been termed 'early death'. It has also been shown that, after immunosuppression either with cyclophosphamide or by thymectomy and irradiation⁴, rabies-infected animals survive longer with decreased incidence of paralysis. It appears that 'early death' also occurs in humans who have been treated (unsuccessfully) with vaccine, with or without accompanying serum therapy^{5,7}, after exposure to rabies. Collectively, these studies suggest that there is a immunopathological component in rabies virus infection. We have therefore investigated a model of lethal rabies infection in immunosuppressed mice and have concluded that B lymphocytes or antibody specific for rabies play a part in the causation of early death. Accordingly the immune response to rabies has a dual role⁸, sometimes favouring survival but sometimes enhancing the disease.

In this study, we used the high-egg-passage Flury (HEP) strain of rabies virus, which causes a limited inapparent infection of the central nervous system (CNS) in adult mice⁹ that can be converted into a fatal infection by immunosuppression¹⁰. This fatal infection can in time be aborted by adoptive transfer of syngeneic immune spleen cells¹¹. Adult BALB/c mice were lethally irradiated by total body irradiation (TBI) from a ¹³⁷Ce source (800–850 rad). TBI and normal mice were infected intracerebrally (IC) on the day of irradiation with 5,000 suckling mouse ICLD₅₀ of HEP virus. As shown in Table 1, untreated TBI mice died due to radiation; but could be protected by normal bone marrow cells devoid of mature T lymphocytes. Normal mice infected with HEP virus survived, but HEP-infected TBI mice died despite immunological reconstitution with bone marrow cells, clearly separating rabies death from that due to radiation.

HEP-infected TBI mice reconstituted with normal spleen cells or cells from donors immunized 30 days earlier (memory cells), died after a mean survival time (MST) of 9 days, but spleen cells from donors immunized 7 days earlier (primary immune cells) conferred complete protection with animals showing no apparent symptoms of the disease.

However, adoptive transfer of spleen cells obtained from donors 3 days after secondary immunization (secondary immune cells) resulted in early death of 28% of the recipients, while the rest survived. The animals that died within 2–3 days after cell transfer did not show any signs of paralysis, typical of rabies, but rather acute symptoms such as convulsions and tonic spasms. Onset of these symptoms was noted only a few hours before death. Histopathological examination of parasagittal sections of the brain from nine of these animals that were either moribund or just dead showed no inflammation, neuronal destruction or other alteration. By contrast, in brains obtained immediately after death from a group of eight mice dying of rabies 13–15 days after infection, foci of necrotic hippocampal cells with minimal inflammation were consistently seen.

To characterize further the immunological requirements for early death, secondary immune spleen cells from syngeneic donors were separated into B- and T-cell enriched populations by passage through a nylon wool column¹² or by treatment with anti- θ antibody and complement. TBI mice infected with HEP

Table 1 Effect of TBI and adoptive cell transfer on HEP virus infections

TBI	HEP	Donor cells	Mortality		
			Early death*	Total	MST
+	—	—	0/10	10/10	9
+	—	Bone marrow	0/15	0/15	—
—	+	—	0/10	0/10	—
+	+	—	0/10	10/10	8
+	+	Bone marrow	0/20	20/20	10
+	+	Normal spleen	0/14	14/14	9
+	+	Immune spleen			
		1° + 7†	0/20	0/20	—
		1° + 30	0/12	12/12	9
		2° + 3	9/32	9/32	3

10–12-week old BALB/c mice were irradiated (800–850 rad, TBI) and on the same day were inoculated with 5,000 suckling mouse ICLD₅₀ of HEP virus. Four days later, these mice were injected intravenously (i.v.) with 10⁷ bone marrow cells or 10⁸ spleen cells from syngeneic donors. MST, mean survival time (days) for mice that died, counting from the 4th day of infection when cells were transferred.

* Indicates mortality within 72 h of cell transfer.

† Indicates primary or secondary immune cells and the time of collection, in days, after last donor immunization.

Table 2 Induction of antibody-mediated death in TBI and HEP virus-infected mice

Immunotherapy	Early death* (%)	Total mortality (%)
B cells	6/27 (22)	100
T cells	0/24 (0)	100
Anti-rabies antibody	6/24 (25)	100
Normal serum	0/12 (0)	100

10–12-week old BALB/c mice were irradiated (800–850 rad) and, on the same day, inoculated with 5,000 suckling mouse ICLD₅₀ of HEP virus. T and B cells were obtained from donors immunized with rabies virus 33 and 3 d before collection of spleen cells; 60 × 10⁶ fractionated viable cells were given i.v. on day 4. Mouse hyperimmune anti-rabies antibody and normal serum were given intraperitoneally in volumes of 0.5 ml each on day 4.

* Death within 72 h of cell or serum transfers.

were given enriched B cells, T cells, mouse anti-rabies antibody or normal mouse serum. The data summarized in Table 2 show that either immune B lymphocytes or rabies-specific antibody, similar to secondary immune cells, can induce early death, whereas T lymphocytes failed to do so. The failure of B or T lymphocytes or antibody to protect is probably due to incomplete immunological reconstitution of these animals^{11,13–15}.

These results clearly demonstrate that early death from rabies virus is mediated by antibody rather than by immune T cells. However, the mechanisms by which the antibody precipitates the disease are not obvious. Although it is well established that anti-rabies antibody plus complement can lyse rabies-infected cells in culture¹⁶, involvement of such a mechanism in early CNS lesions needs further study. This model may lead to further understanding of the contribution of host immunological responses to rabies pathogenesis, and may also be relevant to the pathogenesis of obscure virus-initiated human diseases such as the acutely fatal encephalopathy of Reye's syndrome.

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Monoclonal antibody identifies a new Ia-like (p29,34) polymorphic system linked to the HLA-D/DR region

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The Ia antigens of the mouse are the basis for the genetic control of the immune response. The HLA-D/DR locus is considered to be the human counterpart of the Ia subregion of the murine major histocompatibility complex^{1–4}. The HLA-D/DR antigens are polymorphic, and eight well defined alleles have been identified using alloantisera⁵. More recently, 'supertypic' antigens (MB and MT) have been defined which identify clusters of HLA-D/DR specificities^{6–9}. Little is known about the molecular basis for the cellular and serological polymorphism of the HLA-D/DR antigens, as alloantisera are usually of very low titre and heteroantisera frequently lack monospecificity. We present here the preparation and characterization of a monoclonal antibody which defines a new polymorphic system of the HLA-D/DR region. This and similar antisera should now begin to provide the reagents with which to correlate molecular structure with the functional repertoire of the human Ia-like antigens.

Six-week old BALB/c mice were immunized intraperitoneally with 5×10^6 ascites tumour cells from a patient with Burkitt's lymphoma. Four weeks later, a mouse was boosted intravenously with 5×10^6 tumour cells and somatic cell hybridization was carried out 4 days later by the modified¹⁰ method of Kohler and Milstein¹¹. Splenocytes (1×10^8) were fused in 30% polyethylene glycol with P3/NS1/1-Ag4-1 myeloma cells (2×10^7) and aliquoted into six microtitre plates (Falcon). Aminopterin was added 24 h after fusion, and macroscopic hybridoma colonies became evident between 10 and 28 days in culture.

Preliminary screening identified several hybridoma antibodies with reactivity limited to: (1) the immunizing Burkitt's lymphoma tumour cell, (2) B-cell lines, but not T-cell lines, and (3) 20–25% of Ficoll–Hypaque fraction of peripheral blood mononuclear cells from normal individuals. All cell-surface characterization was done by standard indirect immunofluorescence assay with subsequent analysis of each population by the fluorescence-activated cell sorter (FACS-I, Becton-Dickinson)¹².

Antisera were then evaluated to determine whether their cellular reactivity was similar to that previously described for anti-Ia-like antisera. The peripheral blood of several individuals

was separated, as previously described¹³, into T, B, monocyte and null cell subpopulations. In addition, purified T cells were stimulated with phytohaemagglutinin (PHA) or concanavalin A (Con A). One antibody, designated I-LR1, reacted with >90% of B cells and monocytes, ~25% of null cells and 25% of Con A- or PHA-activated T cells, but was unreactive with freshly isolated or unstimulated cultured T cells. An identical pattern of reactivity with these fractionated cells was found with the previously reported¹⁴ I-1 monoclonal antibody which defines a non-polymorphic Ia-like antigen. In addition, antibodies I-1 and I-LR1 both identify bimolecular glycoprotein complexes composed of subunits of molecular weights 29,000 and 34,000 (see below and Fig. 1) which have been previously shown using heteroantisera to define human Ia-like antigens^{15,16}. Hybridoma I-LR1 was then cloned by limiting dilution and injected into pristane (Aldrich)-primed BALB/c mice to produce immune ascites¹⁷. Monoclonal antibodies I-LR1 and I-1 were then tested on the B cell-enriched mononuclear cells from 50 healthy

Table 1 Reactivity of I-LR1 with homozygous typing cells

Dw	DR	No. HTCs tested*	No. positive with monoclonal antibody	
			I-1	I-LR1
1	1	5	5	4
2	2	9	9	3
3	3	8	8	2
4	4	6	5	0
5	5	2	2	2
6	w6	3	3	2
7	7	5	5	2
10	4	3	3	0
11	7	6	6	3
Total		47	46	18

Cryopreserved HTCs were provided by the National Institute of Allergy and Infectious Disease HTC Typing Panel and by the Histocompatibility Laboratory of the Johns Hopkins Medical School. The NIAID HTC panel represented homozygous cells from several typing laboratories, including: (1) the Sidney Farber Cancer Institute, (2) Memorial Sloan-Kettering Cancer Center, (3) Stanford University, (4) Columbia University, and (5) Leiden. These cells were typed for HLA-A, -B, -C, -DR and -D, and are presented by D typing. They include: Dw1 (BVR, OOS, RM-8W104, CAH and J. Beil); Dw2 (FVV, VN2, KJL, TAL, TI, PA, NOL, LA and VML-8W113); Dw3 (AVL, NR3-8W122, NC-3, TPA-8W123, SFN RW, HRA, 2853-8W119 and KF-8); Dw4 (SFN BN, RJM, MWM, GW-8W132, ER-81128 and 5-0107); Dw5 (NAB-8W142 and 6W7006-8W137); Dw6 (HHK, JGr and Daudi); Dw7 (P. Burk, SFN MA, JB12 and MMF); Dw10 (EM10-8W210, FS-8W212 and 2046-8W213); and Dw11 (DMT, MSA, CA, JVM, J. King and D. Beil). (8W represents the Eighth Histocompatibility Workshop designation of the HTCs.) All analyses were carried out on cryopreserved cells with B cell-enriched fractions isolated by Sephadex G-200 anti-F(ab')₂ chromatography. Cells ($1-2 \times 10^6$) were treated with 0.1 ml of a 1:5,000 dilution of an anti-Ia monoclonal antibody or a 1:5,000 dilution of an unreactive control antibody of a similar immunoglobulin isotype, incubated at 4°C for 30 min, and washed twice. The cells were then reacted with 0.1 ml of a 1:40 dilution of a fluoresceinated goat anti-mouse IgG at 4°C for 30 min, washed twice, and analysed on the FACS-I or Cytofluorograf FC200/4800A. Intensity of fluorescence for I-1 or I-LR1 was determined for 40,000 cells in each population by standard indirect immunofluorescence and compared with the fluorescence of an unreactive isotype identical monoclonal antibody. A displacement of the histogram of the test monoclonal antibody compared with the histogram of an unreactive isotype monoclonal antibody was scored positive. In addition, for each test sample, a quantitative assessment of the number of positive cells was made (number of cells reactive with test monoclonal antibody minus number of cells reactive with the unreactive isotype-identical monoclonal antibody divided by 40,000 total cells tested).

* In addition to B cell-enriched fractions isolated from HTC mononuclear cells, 14 HTCs were transformed by Epstein-Barr virus into lymphoblastoid B-cell lines and the identical patterns of reactivity were found for I-1 and I-LR1 (two lines of each of the following D/DR types: 1,2,3,4,5,7,11).

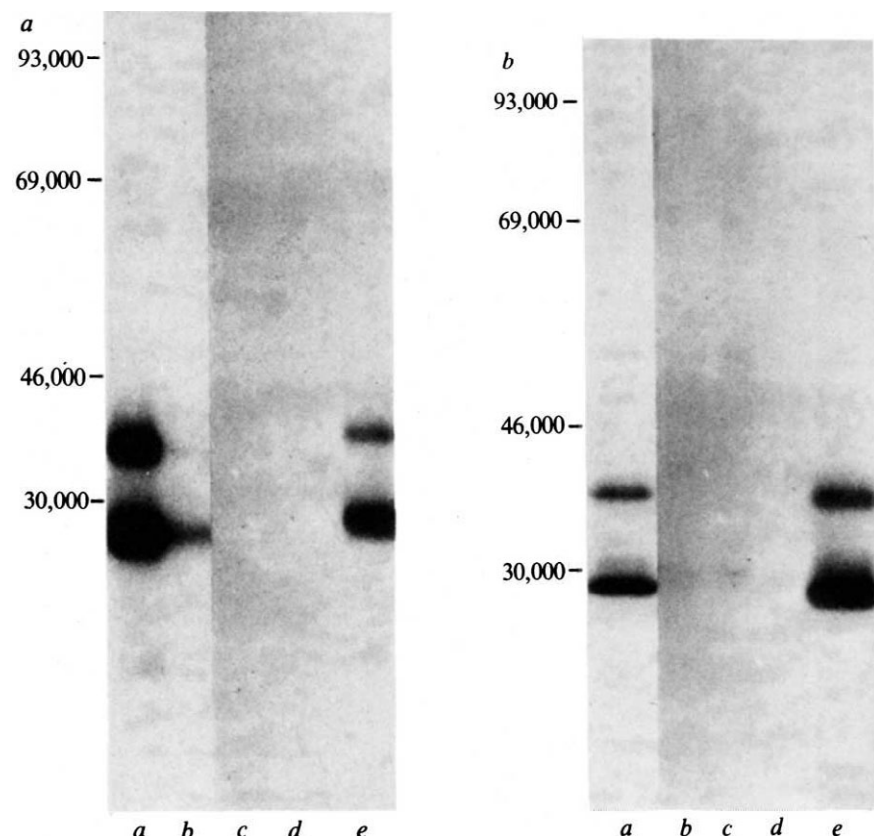


Fig. 1 Serial immune precipitation using monoclonal antibodies I-1 and I-LR1. **A:** *a*, I-1; *b*, I-1; *c*, I-1; *d*, protein A-Sepharose alone; *e*, I-LR1. **B:** *a*, I-LR1; *b*, I-LR1; *c*, I-LR1; *d*, protein A-Sepharose alone; *e*, I-1. The B-lymphoblastoid cell line, Laz 388, was surface-labelled with ^{125}I by the lactoperoxidase method²³. NP40-solubilized membrane extracts were applied to a lentil lectin column and bound glycoproteins eluted with 3% α -methyl-D-mannoside. In conditions of antibody excess, an aliquot of lentil lectin-retained labelled membrane material was incubated three times with I-1 bound to protein A-Sepharose for 14 h at 4 °C. After each extraction, pellets were washed three times in a detergent containing buffer and counted along with a control sample to follow the efficiency of the extraction process. The supernatant was then incubated for 14 h with protein A-Sepharose without added antibody and processed in a similar way to control for any free antibody-antigen complexes which might be present. The supernatant was then incubated overnight with I-LR1 bound to protein A-Sepharose. Similar serial extractions were performed with the I-LR1 antibody. Antigens were resolved by electrophoresis on a gradient polyacrylamide slab gel and visualized by fluorographic processing and autoradiography as described previously²³. Molecular weights are shown.

unrelated HLA heterozygous individuals. Antibody I-LR1 was reactive with the B cells of only 28 individuals, whereas antibody I-1 reacted with all 50, suggesting that the I-LR1 antigen was polymorphic.

To investigate the specificity of I-LR1 further, a panel of homozygous typing cells (HTCs) was examined. As shown in Table 1, I-1 reacted with the B cell-enriched fraction of 46 of 47 HTCs tested, and I-LR1 reacted with only 18 of 47. Moreover, the pattern of reactivity of I-LR1 did not identify a monospecific HLA-D/DR determinant, nor did it identify a previously defined cluster of HLA-D/DR determinants (supertypic). In addition, five separate pairs of HLA-D/DR-identical individuals (pairs including HLA-D/DR 2,3; 4,7; 4,6; and 1,4) displayed reciprocal patterns of reactivity with I-LR1 (for example, D/DR 2,3 reactive, I-LR1 reactive and D/DR 2,3 reactive, I-LR1 nonreactive). This pattern of reactivity on HTCs suggests that I-LR1 defines a new Ia-like specificity.

Additional support for the notion that the I-LR1 antigen was encoded by the major histocompatibility complex was demonstrated by family inheritance studies. As shown in Table 2

(family Kan), I-LR1 reactivity segregates with the maternal 'c' haplotype. This observation strongly suggests that the I-LR1 locus segregates with alleles linked to the HLA complex. Linkage to the HLA-D/DR locus was then demonstrated in families with HLA-D/DR recombinant individuals. As shown in Table 2 (family Sch), the I-LR1 reactivity segregates with the maternal 'c' haplotype. Children 3 and 4 are HLA identical, except at the HLA-D/DR region (see Table 2 legend). B cells from child 3 were reactive with I-LR1, whereas the B cells from child 4 were nonreactive, suggesting that the I-LR1 locus is closely linked to the HLA-D/DR locus. Three additional families (data not shown) provided informative linkage data for I-LR1. For the total of five families (26 of 26 individuals), a determination of the linkage to HLA-D/DR was determined by the lod score method¹⁸, and a very tight linkage to the HLA-D/DR was found ($\theta_{\max} = 0.0$, lod score 6.321). These family studies demonstrate that I-LR1 is linked to the HLA-D/DR locus.

The present study demonstrates that the I-LR1 antibody identifies a polymorphic antigen which shares a pattern of

Table 2 Inheritance of I-LR1 within families

Family identity	Antibody reactivity	Paternal haplotypes ($\frac{a}{b}$)	Maternal haplotypes ($\frac{c}{d}$)	Reactivity with siblings 1 2 3 4 5 6 7 8
Kan	I-LR1	A1, Cw*, B8, Dw3, DR3	A3, Cw-, B18, Dw1, DR1	a b b a b a b
		A3, Cw-, B7, Dw1, DR1	A28, Cw4, Bw35, Dw2, DR2	c d d d c d d
		0	+	+ 0 0 + 0 0 0
Sch	I-LR1	A1, Cw-, B8, Dw3, DR3	A2, Cw5, Bw44(B12), Dw5, DR5	a b a a
		A23, Cw4, Bw44(B12), Dw7, DR7	A3, Cw6, B57(B17), Dw7, DR7	d c c c
		0	+	0 + + 0
Recombinant—Sch family		Child 3	Child 4—recombinant HLA-B/D	
		a A1, Cw-, B8, Dw3, DR3	a A1, Cw-, B8, Dw3, DR3	
		c A2, Cw5, Bw44(B12), Dw5, DR5	c/d A2, Cw5, Bw44(B12), Dw7, DR7	

* A dash indicates that no typing was assignable with present international typing reagents.

cellular reactivity, molecular weight and HLA linkage with those previously described for human Ia-like antigens. However, the pattern of reactivity on HTC and HLA-D/DR-identical heterozygotes suggests that this antibody defines a new Ia-like antigenic specificity distinct from the known DR, MB and MT specificities. Lampson and Levy¹⁹ have recently demonstrated that two monoclonal antibodies defining non-polymorphic Ia-like antigens identify distinct populations of Ia-like (p29,34) molecules. We now have evidence that the p29,34 molecule identified by I-LR1 is distinct from the p29,34 molecule defined by a monoclonal antibody, I-1 (ref. 14), directed against a non-polymorphic Ia-like antigen (Fig. 1). Extraction of radiolabelled membrane material from the B-lymphoblastoid cell line Laz 388 with I-1 monoclonal antibody removes all the p29,34 glycoproteins recognized by that reagent (Fig. 1A, a-e). When this extracted supernatant was tested with I-LR1, clearly defined gp29,34 complexes were still identified. Identical serial extraction using I-LR1 followed by testing with I-1, produced similar results (Fig. 1B, a-e). As complete extraction of the Ia-like complex recognized by I-1 leaves the Ia-like complex recognized by I-LR1 and vice versa, these antibodies seem to identify different Ia-like molecules which are immunologically as well as genetically distinct.

Using N-terminal amino acid sequencing, several laboratories^{20,21} have demonstrated that the DR and Ia-like antisera so far tested define antigens which share a high degree of sequence homology with murine I-E/C antigens. Recently, Goyert *et al.*²², using peptide mapping and limited N-terminal amino acid sequencing, have found that the heavy subunits of the DRw2 and DRw7 antigens are nearly identical but differ significantly from the respective subunit of the DRw5 antigen. They have, therefore, speculated that DRw5 may represent the human counterpart of the mouse I-A subregion antigen. It is intriguing to speculate that the I-LR1 antigen may identify one allele of a new polymorphic system which may be similarly encoded by the human counterpart of the murine I-A locus. The precise relationship of the I-LR1 antigen to the murine Ia antigens will require further biochemical investigations.

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A cyanine dye distinguishes between cycling and non-cycling fibroblasts

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Cellular proliferative activity has previously been determined by measuring the incorporation of radiolabelled nucleotides or by visual inspection of cellular morphology. Although two flow cytometric methods^{1,2} have recently been developed which can distinguish cycling from non-cycling cells, both have serious disadvantages. One method requires uptake of a substantial amount of BUdR¹, limiting its usefulness for *in vitro* systems. The other method utilizes RNA/DNA content differences² but its successful application has proved cell-type dependent. We have now used the findings that the cell membrane is more highly polarized in resting than in proliferating cells³ and that cyanine dyes carrying a delocalized positive charge enter live cells to an extent that depends on the cell membrane potential⁴, to develop a method of distinguishing between cycling and non-cycling cells. The greater the membrane polarization, the greater is the concentration of dye within the cell. At high concentrations, the dye molecules aggregate and their fluorescence is quenched⁵. Thus, for a given external dye concentration, cells of different membrane potential would accumulate different amounts of fluorescent (non-aggregated) dye. Using fibroblasts in culture conditions chosen to provide various models of cycling and non-cycling cells, we found that fluorescence intensity with the dye 3,3'-diheptyloxycarbocyanine (Di-O-C₇(3))⁶ was consistently greater in the former than the latter.

The human foreskin fibroblasts used in these experiments show virtually no mitotic activity once confluent. In the first

Table 1 Changes in fluorescence intensity of cycling and non-cycling fibroblasts with dye concentration

Dye concentration (M)*	Wound margin†	Confluent region†	Ratio‡	Background§
2.5×10^{-6}	246.0 ± 9.40	183.0 ± 12.7	1.34 ± 0.11	4.80 ± 0.32
1.0×10^{-6}	290.0 ± 12.1	112.0 ± 6.7	2.59 ± 0.35	5.50 ± 0.30
5.0×10^{-7}	258.0 ± 16.5	46.8 ± 3.1	5.51 ± 0.51	2.55 ± 0.03
2.5×10^{-7}	104.0 ± 7.50	22.9 ± 2.2	4.56 ± 0.54	2.62 ± 0.15
1.0×10^{-7}	70.7 ± 8.90	13.3 ± 1.9	5.32 ± 1.01	1.93 ± 0.16
5.0×10^{-8}	21.1 ± 1.34	8.34 ± 0.9	2.53 ± 0.42	1.68 ± 0.20

Slides were analysed with a Zeiss Axiomat photometer. Fluorescence was excited with epi-illumination from a 100-W mercury arc lamp; the exciter filter was a BP 450-497, the dichroic mirror an FT 510 and the barrier filter an LP 526. Areas of interest on the slide were initially identified with a $\times 10$ or $\times 25$ objective under bright-field phase. Measurements of cytoplasmic fluorescence from selected cells were made with a $\times 50$ 0.95 NA dry objective and an effective photometer aperture of 4- μ m diameter. Fluorescence collected by this aperture was detected by a Hamamatsu R-928 photomultiplier and the output pulses from the tube were detected and counted by a Princeton Applied Research Corporation Model 1105/1120 ratemeter/discriminator. Fluorescence intensity measurements were made immediately after opening the fluorescence excitation shutter. Although some fading or enhancement of fluorescence was observed over a 30-s period, the rate of change was sufficiently low to permit reproducible measurement of fluorescence intensity, as indicated by the standard deviations.

* Di-O-C₇(3); see Fig. 1 legend for a description of the staining method.

† Thousands of photon counts per cell; expressed as mean $\pm$ s.e. ($n = 15$) after subtraction of indicated background.

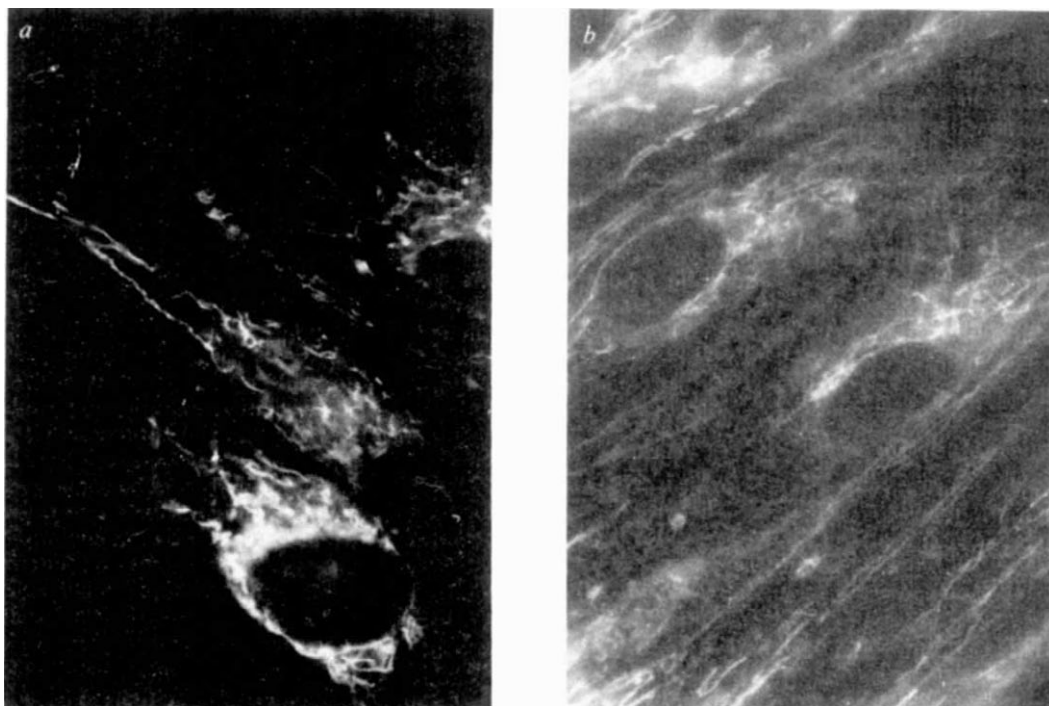
‡ Wound margin mean/confluent region mean.

§ Thousands of photon counts as measured in the centre of the wound away from all cells; expressed as mean $\pm$ s.e. ($n = 4$).

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Fig. 1 Fluorescence distribution in fibroblasts stained with the cyanine dye, Di-O-C₇(3). Human foreskin fibroblasts were wounded and stained with 1.0×10^{-7} M Di-O-C₇(3) 4 h after wounding. Fluorescence photomicrographs were taken using a $\times 100$ oil immersion objective and identical exposure times for cells at the wound margin (a) and cells in the confluent region of the same coverslip (b). Maximal definition of the fibrillar structures shown was observed at a stain concentration of 1.0×10^{-7} M. At 5×10^{-7} M more diffuse cytoplasmic staining was observed, although the intensity ratio for wounded and confluent cells was not significantly different at the two concentrations (Table 1). Di-O-C₇(3) (synthesized by A.S.W.) exhibits maximum absorbance at 488 nm (in ethanol) and maximum fluorescence at 504 nm. Stock dye solutions were prepared by adding ~ 1 mg of crystalline dye per ml of ethanol. Stock concentrations were assessed spectrophotometrically, based on an extinction coefficient of 1.49×10^5 M⁻¹ cm⁻¹. Stock solutions were stored for up to 2 months in foil-wrapped tubes at 4 °C. The human skin fibroblasts (donor HS0001, Naval Bioscience Laboratory, Oakland) were used for passages 1–5 and grown at 37 °C on coverslips placed in sealed Leighton tubes containing 10% CO₂. The growth medium consisted of DMEM supplemented with 10% fetal calf serum, penicillin (0.2 units ml⁻¹) and streptomycin (0.2 µg ml⁻¹). Growth medium was changed every 3–4 days. Wounding of monolayers was accomplished by scraping a strip of confluent cells from a coverslip using a modified metal microspatula and sterile procedures. Tubes containing scraped coverslips were returned to the incubator until staining. The cells were stained using the following procedure. Culture tubes were removed from the incubator and the growth medium was removed by aspiration. The slides within the tubes were then rinsed twice before staining. The rinse solution was Dulbecco's phosphate-buffered saline supplemented with 4.5 g l⁻¹ glucose. The staining solution consisted of the above salt solution plus an appropriate concentration of dye. The dye was allowed to equilibrate with the cells for 15 min at room temperature in the dark. Coverslips were then removed from the tubes with forceps, wet mounted using staining solution and sealed with paraffin. Photographs were taken with the 35-mm camera built into the Zeiss Axiomat. Kodak type EH-135 film was exposed for 5 s and developed at ASA 160.



system, we used contact-inhibited confluent cultures grown on glass coverslips as a model for the non-cycling state. A 'wound' scraped in a confluent monolayer releases the cells at the wound margin from contact inhibition³, thus providing both cycling and non-cycling cells on the same coverslip. To determine the maximum fluorescence difference between active and quiescent cells, we examined monolayers at a variety of dye concentrations 4 h after wounding (Fig. 1, Table 1). Table 1 shows that cycling cells were more fluorescent than non-cycling cells over a 50-fold range of dye concentration, with the intensity ratio varying from 1.5 to 5. As the maximum value of the intensity ratio was obtained with 5×10^{-7} M dye, this concentration was chosen for further studies.

The viability of cells in dye was assessed by a double staining experiment using ethidium bromide (1×10^{-6} M) as a measure of the cells' ability to exclude ethidium bromide; dead cells concentrate this dye within their nuclei, while viable cells remain unstained⁷. The cells remained viable and excluded ethidium bromide for at least 2 h in the presence of 5×10^{-7} M cyanine dye, indicating that Di-O-C₇(3) itself is not toxic in these

experimental conditions. In addition, absence of ethidium bromide staining in the cells at the wound margins indicates that cells brightly stained with cyanine dye were not damaged by the wounding process. Thus, the intensity differences shown in Table 1 are not the result of differences in viability of the cell populations.

Intensity differences were also observed in our second model system, using fibroblasts grown to different cell densities. Non-confluent and confluent cells on different coverslips were stained and measured using the previously outlined protocol. As Table 2 demonstrates, the non-confluent (cycling) cells stain more intensely than the confluent (non-cycling) cells.

Table 2 also summarizes the results obtained in the third model system, in which non-confluent cells were incubated for various periods of time in non-permissive medium (0.5% fetal calf serum), in which they become essentially non-cycling cells⁸, and measurements were made as previously described. After 6 days in this media, the cell fluorescence approached that of confluent contact-inhibited cells, although the monolayer was still subconfluent. Visual inspection of cells in low-serum medium did suggest a low level of mitotic activity.

To determine the time required for cells at the edge of a wound in a confluent layer to become significantly brighter than cells in the interior, six confluent monolayers were simultaneously wounded by scraping, returned to media and at various times later stained with 5×10^{-7} M dye for 15 min and then assayed for fluorescence intensity. The results of this experiment (Table 3) may be summarized as follows. (1) Fluorescence changes in the cells at the wound margin were noted as soon as measurements could be made, that is, within 18 min. (2) Maximal fluorescence intensity of the bright cells occurred within 18 min and a constant fluorescence ratio of wounded to confluent cells was then maintained for at least 1 h. (3) The per cent of bright cells at the wound margin increases with time and

Table 2 Effect of growth medium on fluorescence intensity in cycling and non-cycling fibroblasts

Cell density	Growth medium*	Fluorescence intensity†
Confluent monolayer	Permissive	1.00 ± 0.066
Nonconfluent monolayer	Permissive	2.89 ± 0.18
Nonconfluent monolayer	Non-permissive, 52 h	1.71 ± 0.16
Nonconfluent monolayer	Non-permissive, 6 days	1.15 ± 0.084

* Permissive = Dulbecco's modified Eagle's medium (DMEM) + 10% fetal calf serum; non-permissive = DMEM + 0.5% fetal calf serum.

† Means $\pm$ s.e. all expressed relative to the fluorescence intensity of the confluent monolayer in permissive medium measured after exposure to 6×10^{-7} M Di-O-C₇(3) as described in Fig. 1 legend.

Table 3 Changes in fluorescence of cycling and non-cycling fibroblasts with time after wounding

Time after wounding (min)	Wound margin	Confluent region	Ratio	Background
3	272 ± 25.3	55.1 ± 4.2	4.94 ± 0.39	1.22 ± 0.06
30	197 ± 15.0	42.5 ± 4.3	4.64 ± 0.59	3.48 ± 0.26
60	204 ± 16.1	46.1 ± 3.6	4.42 ± 0.49	3.35 ± 0.32

The cells were stained with 5×10^{-7} M Di-O-C₇(3) as described in the text at the indicated time after wounding. Staining required a 15-min incubation in dye at room temperature in the dark. Parameters measured were as described in Table 1 legend.

bright cells are eventually noted several cell layers into the confluent monolayer. Mitotic figures and rounded cells are observed at later times.

It is interesting to speculate on the mechanism by which the dye reflects changes in the cycling state of the cells. One possibility is that the dye stains cell cytoplasm relatively non-differentially. Observed intensity differences would then be merely the result of differences in cell size and shape between the two cell populations. Two observations oppose this interpretation. First, microscopic analysis suggests that the size and shape differences between bright cells at the wound margin and those in the confluent monolayer are minimal, especially at early time post-wounding. Second, the intensity ratio of bright to dim cells is concentration dependent (Table 1). If the fluorescent intensity difference merely reflected differences in cell size, the ratio of intensities should remain fairly constant, independent of dye concentration.

A possible mechanism for the observed fluorescence difference is that the dye stains some cytoplasmic structure. Figure 1 shows that the dye does not stain nuclear structures and is localized in discrete filamentous structures within the cytoplasm. Comparison of the morphology of staining in Fig. 1 with that obtained by Johnson *et al.*⁹ using rhodamine 123 suggests that these structures might be mitochondria. Cyanine dyes have been used to stain isolated mitochondria and seem to respond to membrane potential in such systems¹⁰. The timed wound experiments (Table 3) indicate that detection of cycling cells can be accomplished considerably earlier than is possible with methods dependent on DNA synthesis. These results also suggest that some cell parameter—perhaps the cellular or mitochondrial membrane potential—changes very soon after the cells are triggered to begin cycling.

Finally, the intensity differences reported here are large enough to allow good discrimination even in cell populations which have fairly broad fluorescence distributions. Such differences should also make the detection of even relatively small cell populations possible using quantitative techniques for single-cell analysis and sorting¹¹. Di-O-C₇(3) staining therefore promises to provide a fast, easy and sensitive method for distinguishing cycling cells from quiescent cells.

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Localization of pacemaking activity in early embryonic heart monitored using voltage-sensitive dye

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Early in cardiogenesis, heart primordia are brought together at the midline and fuse with each other progressively caudally—this results in the formation of the primitive tubular heart which begins beating spontaneously at the middle period of the 9-somite developmental stage in the chick embryo¹. However, in these very early stages of development, the myocardial cells are small and technically difficult to impale with microelectrodes; thus electrophysiological studies on the very early embryonic heart are rare^{2,3}. Recently, potential sensitive dye-related absorption signals have provided a new method for monitoring spontaneous action potential activity in the early embryonic heart⁴⁻⁷. This technique is based on the observation that changes in potential across membrane(s) stained with certain voltage-sensitive dyes are accompanied by changes in their optical properties (absorption, fluorescence, and/or birefringence)⁸⁻¹⁰. Using absorption signals, we have already demonstrated in embryonic pre-beating chick heart in the 7-8-somite stages, the occurrence of action potential activity^{4,5}, development of pacemaker potential^{6,7} and cardiac rhythm generation⁷. With this method, originally introduced to record neuronal activity in invertebrate ganglia^{11,12}, many cells or portions of the preparation can be monitored simultaneously. Accordingly we have expanded the optical recording apparatus to monitor simultaneously spontaneous action potentials from five portions of an early embryonic heart, and report here experiments carried out on the embryonic hearts of chicks (white Leghorn) at the 7-11-somite developmental stages, corresponding to 25-35 h of incubation. The hearts attached to the embryo were stained with a merocyanine-rhodanine dye (NK2761) as a potentiometric probe. This dye is an analogue of Dye XVII⁹ or Dye XXIII¹⁰.

Figure 1 shows simultaneous recordings of spontaneous optical signals from an 8-somite embryonic heart. The circles overlaid on the view of the embryonic heart in this figure are drawn to scale, indicating the field of optical recording and the relative positions of the light guides. Traces 1, 2 and 4 were obtained from the ventricular region, and traces 3 and 5 from the unfused atrial levels. In the preparation shown in Fig. 1b, both size and shape of each spike in individual traces are approximately uniform, which suggests that the action potentials of active cells in the field of optical recording were well synchronized. However, as shown in traces at the higher sweep speed, there were obvious differences in time course of action potentials from the five different regions; the action potentials in the five different regions were synchronized and there was a small delay between spike 5 and that in the other regions. It is thus likely that the delay corresponds to spreading time of the action potentials. At the 7-8-somite developmental stages, the action potential was usually first detected in the left atrial primordium, but sometimes in the right primordium.

Figure 2 shows experiments demonstrating the spatial distribution of spontaneous action potential activity in the 9-11-somite embryonic hearts. In the preparation shown in Fig. 2a, the action potential first appeared in the left atrial portion (trace 5), and action potentials in other portions (traces 1-4) followed those in the left atrial portion after a delay of 40-150 ms. Furthermore, the light guides were positioned close together in a series on the left portion of a 9-somite embryonic heart. In this experiment, the action potential probably occurs first in the left

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atrial level and then spreads progressively in rostral and caudal directions. As shown in Fig. 2b, an action potential also appeared first in the left atrial region in the heart of the 10-somite embryo. Thus, it is reasonable to assume that although both the right and left atria have intrinsic pacemaking abilities, the cardiac rhythm as a whole is governed by the left atrium as the embryo develops from the 7- to the 10-somite stage.

From experiments on the pulsation rate, Patten¹³ suggested that the cardiac pacemaker at the developmental stage of initiation of heartbeat is the atrium. Using microsurgical techniques, DeHaan¹⁴ showed that the earliest pacemaker was on the left

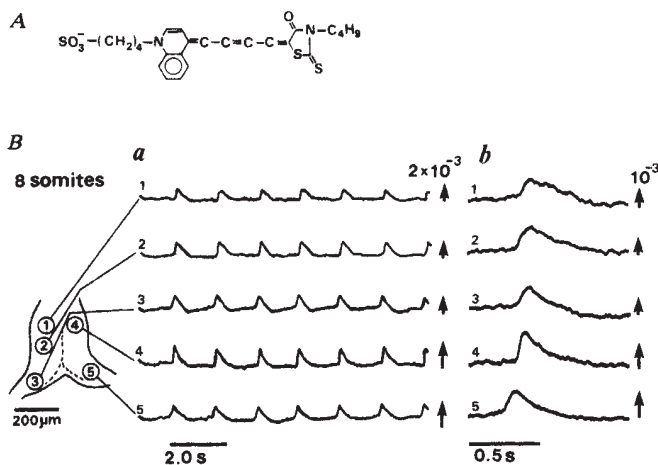


Fig. 1 A, Chemical structure of a merocyanine-rhodanine dye (NK2761, produced by Nippon-Kankoh-Shikiso Kenkyusho Co.) used as a potentiometric probe in our experiments. The cation associated with the dye is sodium. Voltage clamp experiments in squid giant axons gave good evidence that the absorption signals of merocyanine-rhodanine dyes were linearly related to membrane potential change, and that the optical response followed the voltage change with a time constant of $<20 \mu\text{s}$ (refs 9, 10 and L. B. Cohen, personal communication). B, Spontaneous absorption signals detected simultaneously from five regions in an 8-somite embryonic heart. After the splanchnopleure was carefully peeled off, the isolated embryos were incubated for 15 min in bathing solution containing 0.05 mg ml^{-1} of the dye. After incubation, the dye solution was washed out. The dye was bound to the epi-myocardium cells. The solution used to bathe the embryos was 138 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.5 mM MgCl₂, and 10 mM Tris-HCl buffer (pH 7.2), and was equilibrated with air and allowed to warm to $\sim 37^\circ\text{C}$. This solution was used in most experiments, except those shown in Figs. 2B and C. To measure the absorption of light by the area of interest in the dyed embryonic heart, a $\times 50$ enlarged image of the heart was formed with a $\times 20$, 0.46 n.a. (numerical aperture) objective and a $\times 2.5$ photographic eyepiece, using an Olympus-Vanox microscope (AHB-LB-1, Olympus Optical Co.). Five light guides were used to carry the light from the image of the embryonic heart to each photodiode (Bell and Howell, 509-10). The tips of these guides (4.0 mm in diameter) were positioned over the image of the embryonic heart stained with the dye. The measuring beam was produced by a JC 24V/300W halogen-tungsten-filament lamp (Kondo Sylvania) powered by a current regulated power supply (PAD 35-20L, Kikusui Electronics); the incident light was passed through an interference filter with a centre wavelength of 700 nm and a width at half-height of 11 nm (1F-S, Vacuum Optical Japan). The signals in B were eliminated by illumination with white light, or at 620 nm where the action spectrum of the absorption signal of the merocyanine-rhodanine dyes is zero^{6,7}. Thus, the optical signals shown resemble the spontaneous action potentials in the embryonic heart. The empirical criteria required to distinguish between optical signals from an action potential and those corresponding to movement artefacts have been described previously⁵. In experiments where a relatively low light intensity and brief duration were used, photodynamic damage and dye bleaching were not serious. The fractional absorption change ($\Delta A/A_0$) is related to the fractional change in transmitted intensity (T); evidence presented below indicates that a decrease in intensity was due to an increase in absorption. $\Delta A/A_0$ is equal to $-\Delta T/(T_{\text{before}} - T_{\text{after staining}})$ ⁶, ($\Delta T/T_{\text{after}} = \Delta I/I_0$). Here, the transmittance calibration bar is indicated. The traces were detected in a single sweep at 37.6°C , and the oscilloscope (Tektronix 5113 Dual Beam Storage Oscilloscope) was set to give a coupling time constant of 1.5 s. The outputs were finally filtered by a simple RC low-pass filter (time constant $\sim 10 \text{ ms}$). Positions 1, 2 and 4 are from the ventricular region, and positions 3 and 5 on the atrial unfused primordia. The field of optical recording encircled in the figure is estimated as 0.005 mm^2 . Traces b were obtained at the higher sweep speed. The embryonic heart is shown in ventral view.

side, and that it shifted to the sinoatrial region only later. Moreover, Van Mierop² was able to record the earliest action potentials in the left atrial region of the 11-somite beating heart, and in the left atrial primordium in the 8-somite embryo, before contractile activity had begun. Indeed, his transmembrane-potential records are remarkably similar to our optical recordings (Fig. 1b). As development proceeded to the 11-somite stage (Fig. 2c), the portion in which the action potential first occurred shifted caudally along the atrium to the sinus primordium.

In embryonic chick hearts past the 13-somite stage, pacemaker potentials were detected electrophysiologically using microelectrodes^{15,16}. We have optically demonstrated differentiation and development of the pacemaker potential during the 8–9-somite stages^{6,7}. At the 8-somite stage, absorption signals resembling the pacemaker potential are widely distributed in the embryonic hearts but by the beginning of the 9-somite stage these signals are localized at the atrial level¹⁷.

In the present experiment, the cardiac-type signals were obtained from the ventricular region and the pacemaker-type ones from the atrial region. The localization of the latter is consistent with the results obtained from experiments on the time course of action potentials, and shows clearly that the pacemaking area is situated in the atrium region at the 9-somite stage.

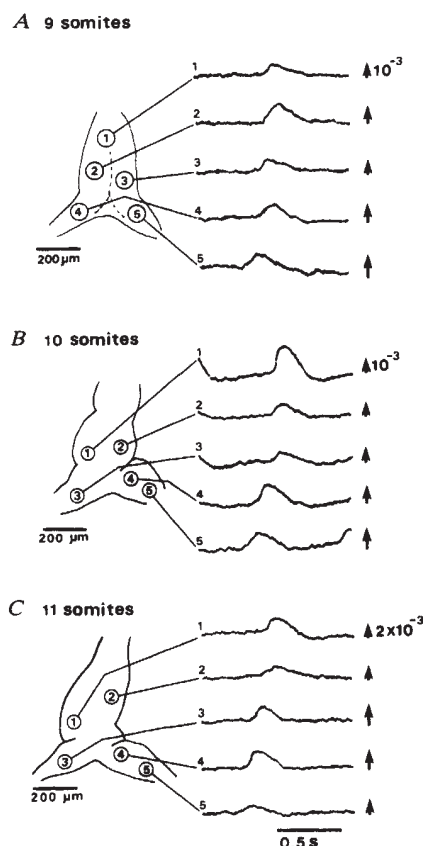


Fig. 2 Simultaneous absorption measurements in five different portions in 9-(a), 10-(b) and 11-(c) somite embryonic chick hearts. Light-guides 1, 2 and 3 were positioned over the image of the ventricular region, and 4 and 5 over the image of the atrial unfused regions in the experiment shown in A. This preparation was obtained just before initiation of the heartbeat⁵. In B and C, traces 1 and 2 were obtained from the ventricular region, 3 and 4 from the atrium, and the position of trace 5 corresponds to sinus primordia. At these stages, the hearts are bent to the right and are pulsating. To reduce movement artifact due to pulsation, we used a hypertonic solution made by increasing NaCl concentration up to 276 mM. In this solution, contraction was reduced. However, such hearts are electrically excitable⁶, as is the case in striate muscle¹⁸. Thus, it is not surprising that no signal was observed by illumination with white light or at 620 nm. It seems likely that differences in the signal size among the different positions are due to tissue thickness and density of the active membrane⁶. Experiments were carried out at 37.3°C (A), 38.3°C (B) and 36.7°C (C).

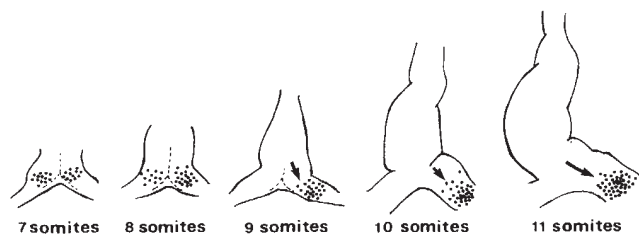


Fig. 3 Maps summarizing the location of the pacemaking area in the embryonic chick hearts throughout early phases of development, from the 7- to the 11-somite stage. Depth of dotting represents the degree of priority of pacemaking. The arrows indicate the direction of translocation of pacemaking area.

On the basis of these results, we constructed a rough developmental map of regional localization of pacemaking areas during the early phases of cardiogenesis (Fig. 3). The possibility exists that pacemaker cells are situated originally in the cardiac primordia just before and after they fuse in the midline. It seems that as the primitive tubular heart is formed, the pacemaking area which possesses the highest intrinsic rhythmicity, is localized mainly in the left atrial primordium and subsequently in the sinus primordium. With further development of the heart, the

pacemaking cells of the sinus primordium probably become organized into nodes and bundles while maintaining their original relationship with the surrounding musculature.

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Direct communication between axons and sheath glial cells in crayfish

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Evidence for the transfer of molecules as large as proteins from glial or Schwann cells to axons has frequently been reported¹⁻⁸. This phenomenon is well supported in crayfish^{3,4,8} and squid⁵⁻⁷ but there is some doubt about its existence in vertebrates⁹. In invertebrates most of the data support the transfer from glial cells to axons, but little is known about movement of molecules in the opposite direction. However, Grossfeld *et al.*¹⁰ recently reported data on the passage of a fluorescent dye (Lucifer yellow CH, molecular weight (MW) 476) from a crayfish median giant axon to all glial cells of the adaxonal layer. Little is known of the mechanism by which molecules are exchanged between axons and glial cells, but the bidirectionality of the phenomenon in crayfish axons suggests that channels or pores for direct intercellular communication may exist. In most cells, direct communication is provided by gap junctions¹¹ but these structures have never been seen between axons and glial cells of crayfish¹²⁻¹⁴. Indeed, regions of plasma membrane specialization have been reported¹³, but they are structurally different from gap junctions. I describe here pore structures, observed between 51 axons and glial cells of six crayfish spinal cords, which may represent the means of communication between these cells.

In crayfish, *Procambarus clarkii*, anaesthetized by cooling, the sternal artery was cannulated using polyethylene tubing and perfused with 50-100 USP units of heparin diluted in 10 ml of Van Harreveld's salt solution¹⁵, followed by 40-60 ml of 3-6% glutaraldehyde solution buffered to pH 7.4 with 0.1 M (Na, K) phosphate at room temperature¹⁴. The chain of abdominal ganglia was removed from the animal, washed briefly in 0.2 M phosphate and post-fixed for 2 h in 2% osmium tetroxide buffered with phosphate to pH 7.4 at room temperature. Dehydration was in graded alcohol and embedding in Epon.

Electron microscope examination of thin sections through axons of the abdominal ganglia often show regions where axonal

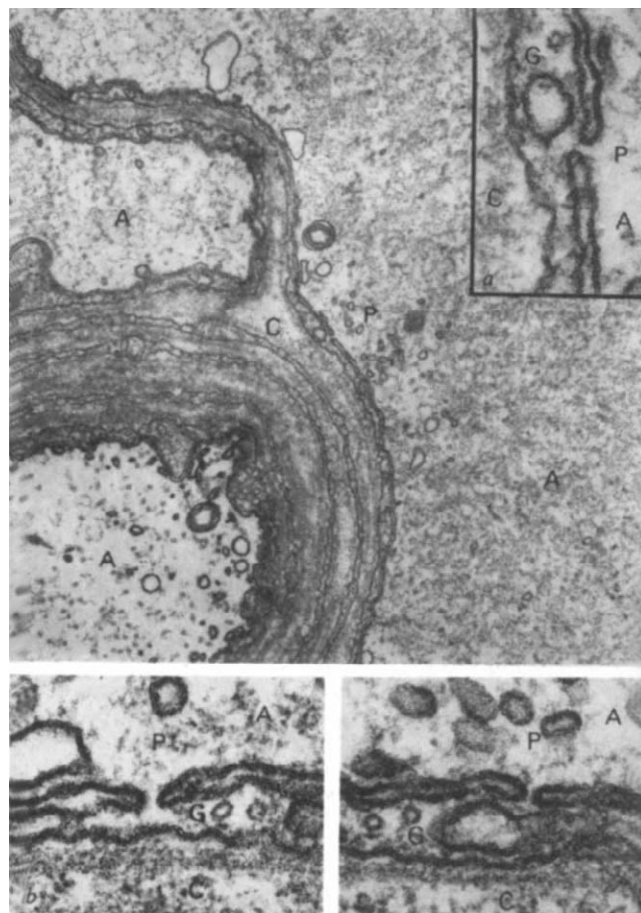


Fig. 1 Thin section through nerve fibre of a crayfish abdominal ganglion. Axonal and glial cell plasma membranes fuse with each other to form a well defined pore (P). In the region of the pore, tubules of endoplasmic reticulum are aggregated. Inset a is an enlargement of the pore shown in the low power micrograph. Insets b and c show similar pores in other axons. A, axon; G, glial cell; C, connective tissue. $\times 18,000$; insets $\times 77,000$.

and glial cell plasma membranes fuse with each other to create well defined pores between axoplasm and glial cell cytoplasm (Fig. 1). Several such pores are usually grouped in the same region. In these regions there are always aggregates of endoplasmic reticulum tubules and other membrane structures (Fig. 1). The pores generally have 15–20 nm diameters but larger pores are also seen (Fig. 1, insets).

Pores of this size are expected to allow wide communication between axoplasm and glial cell cytoplasm. It seems unlikely that such structures are artefacts of the preparative procedure, as the membranes and other cellular structures of these perfused samples were always extremely well preserved. Furthermore, if the pores were artefacts, one would expect to see them anywhere and not just at regions of endoplasmic reticulum accumulation. Indeed the existence of pores between axons and Schwann cells of invertebrates seems reasonable from a theoretical viewpoint, because only passageways of this size could allow exchange of molecules such as RNA³ and proteins as heavy as 200,000 MW (refs 5, 6).

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Potassium channels in the nodal membrane of rat myelinated fibres

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Following some preliminary reports^{1,2}, mammalian fibres from rabbit³ and rat^{4,5} have recently been successfully studied in detail by means of the voltage clamp. The early transient or sodium conductance system was found to be similar to that in frog and squid axons. However, the delayed conductance or potassium currents were found to be negligible^{4,6}. Only after chemical and osmotic manipulations, which were said to expose channels buried under the myelin, did Chiu and Ritchie⁷ find delayed currents in rabbit fibres. If confirmed, this would mean that the membrane conductance system of mammalian fibres is so different from that of invertebrate and amphibian axon models as to make the data base gathered from amphibian myelinated fibres (frog and toad) and invertebrate giant axons (squid and myxocela) irrelevant to human and other mammalian fibres. However, we show here that it is possible to find in the normal nodal membrane of rat myelinated fibres potassium currents that flow through channels which are similar in many respects to those found in the frog node or squid axons.

Single myelinated motor fibres isolated from the sciatic nerve of rats were voltage clamped following the procedure of, and using the basic apparatus described by Nonner⁸. The voltage

clamp command potentials as well as the membrane current sampling rates were under digital computer (NOVA 800) control⁹. Holding potential (V_H) was adjusted to give a 70% sodium conductance inactivation. Membrane potential (V_M), which was defined at the end of each experiment by destroying the nodal membrane, averaged -78 ± 5 (s.d.) mV in 13 preparations. All currents were corrected for leakage (I_L) by computing the leak conductance from the inward currents elicited by membrane hyperpolarization. It was assumed that the leakage current–voltage relationship is linear and that $I_L = 0$ at the resting potential. The node was externally perfused with mammalian Ringer's solution. Potassium currents were measured in the presence of 300 nM tetrodotoxin (TTX). The composition of the internal solution was controlled by diffusion through the fibre's ends which were cut 400–700 μ m away from the node¹⁰. Currents were converted to densities (mA cm^{-2}) assuming the nodal area to be 50 μm^2 (ref. 9).

Current tracings obtained in TTX Ringer at normal holding potentials, V_H , are shown in the insert of Fig. 1a. The tracings show a large instantaneous outward current jump (I_{inst}) followed by a delayed current component. I_{inst} was measured by extrapolating (on semilogarithmic scale) the initial position of the current⁷ tracing, beyond the capacity transient, to zero time.

The instantaneous current shows a linear $I_{\text{inst}}-V$ relationship, while the steady-state current, $I_{\text{ss}}-V$ curve shows some rectification and is very similar to that of the frog node (Fig. 2). Note that following the relatively rapid increase of the current in the first 3 ms there is a steady slow increase in current. This additional slow activation can be best demonstrated by the change of slope of the $\log(I_{\infty} - I_t)$ versus t curve as seen in the inset of Fig. 3b. In the example given, the initial time constant, τ_n , is 0.53 ms (this value is obtained after 'peeling off' the second time constant) and the slower time constant, τ_s , is 1.36 ms. The corresponding single τ at $V_H = -71$ mV is 0.56 ms, that is, τ_n is not altered by depolarizing V_H . Shifting V_H in the hyperpolarizing direction ($V_H = -100$ mV) has very little effect on the instantaneous current and produces a 15% increase in the delayed currents (open symbols in Fig. 1a). However, a relatively small (9 mV) shift of V_H in the depolarizing direction has a marked effect on the current tracings (Fig. 1b): they become square and are thus similar to those described by Chiu *et al.*⁶ and Brismar⁴. The squaring of the current traces, which was completely reversible, results mainly from an increase of the I_{inst} values to approach those of the steady state (I_{ss}). I_{ss} remains unchanged for depolarizations up to +10 mV, beyond which the currents generated at $V_H = -71$ mV are reduced by 10–15%. Note, however, that most of this reduction is due to the disappearance of the slow activation (see Fig. 1b). This disappearance or inactivation of the slow process obviously also contributes to the 'squaring off' of the currents.

Figure 2 compares the steady-state $I_{\text{ss}}-V$ relationship in the frog and rat. The shape of the curves obtained by means of the same voltage-clamp setup and procedure is clearly very similar, although the frog curve shows more rectification.

Moreover, in both cases the zero-current voltage cross-over point is shifted from around rest in normal Ringer's towards zero in high external $[\text{K}^+]$, indicating that the current is carried mainly by K^+ ions. Note the difference between the effect of high $[\text{K}]_o$ on the two preparations: it increases the potassium conductance, g_K , by about 50% in the frog and reduces g_K by 30% in the rat. The assumption that K^+ ions are the main charge carriers in the delayed channels is strengthened by the fact that the axoplasm in our fibres was in equilibrium with isotonic KCl solution. Therefore, the only membrane permeable ions in the axoplasm must be K^+ and Cl^- (ref. 10). As the chloride concentration remained unchanged, the large shift of the reversal potential indicates that the chloride permeability is relatively low. This low chloride permeability which is also typical of the frog node¹¹, indicates that the outward currents we are dealing with must be mainly carried by K^+ ions.

As reported by Chiu⁶ and Brismar⁴, we also find that external tetraethylammonium (TEA) only partly attenuates I_{ss} even at

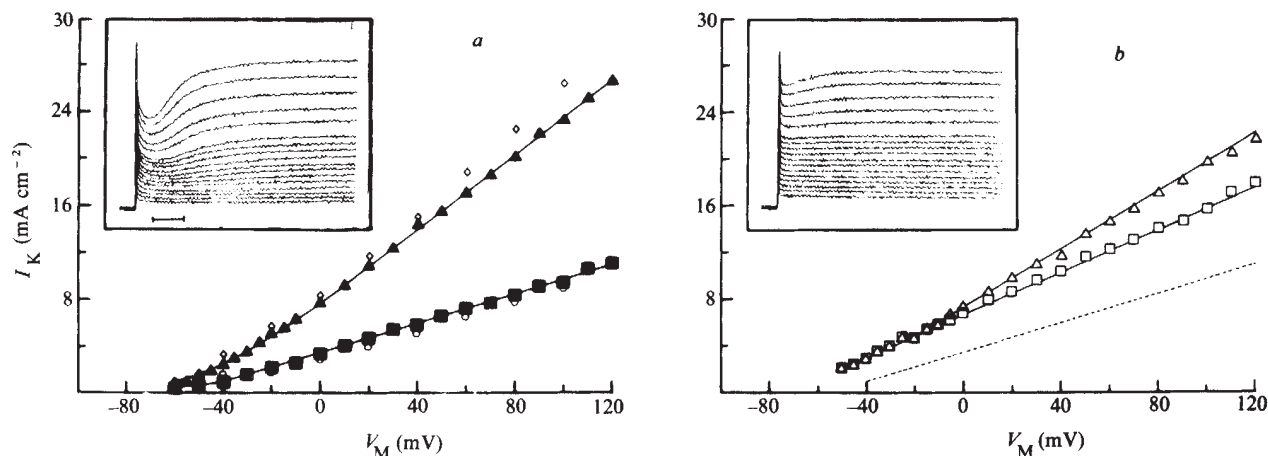


Fig. 1 Steady-state and instantaneous current-voltage relationships at various holding potentials (V_H). All currents corrected for leakage. Test solution: Ringer TTX. Temperature 20 °C. *a*, $\blacktriangle$, Steady-state currents at $V_H = -80$ mV. $\blacksquare$, Instantaneous currents at $V_H = -80$ mV. $\diamond$, Steady-state currents at $V_H = -100$ mV. $\circ$, Instantaneous currents at $V_H = -100$ mV. Inset illustrates the recorded membrane currents as a function of time for different depolarizing pulses. The potential increment between the pulses was 5 mV from 15 to 60 mV and 10 mV from 60 to 120 mV. Calibration: horizontal bar = 0.8 ms. *b*, $\blacktriangle$, Steady-state currents at $V_H = -71$ mV. $\square$, Instantaneous currents at $V_H = -71$ mV. The interrupted line gives the instantaneous currents generated by the same fibre at $V_H = -80$ mV. Inset illustrates the recorded membrane currents as a function of time for different depolarizing pulses. The pulse amplitudes are the same at those of *a*, as is the time scale.

relatively high concentrations (10 mM TEA attenuates I_{ss} by only 30%). I_{inst} was practically unaffected by TEA.

Figure 3*a* illustrates the voltage dependency of the normalized delayed channel steady-state conductance parameter, n_{∞}^* , which was computed using the following relationship:

$$n_{\infty}^* = I_{ss} / \bar{g}_K (V_M - V_K) \quad (1)$$

where $\bar{g}_K$ is the maximal membrane potassium conductance, V_K is the delayed channel reversal potential and x is the power of n in the Hodgkin-Huxley axon model. It is seen that near the resting potential ($V_M = -60$ mV) the potassium conductance, as determined by n_{∞}^* , is far from zero for both $x = 4$ (circles) and $x = 1$ (triangles). This is in contrast to the frog node or squid, where for the experimentally determined $x = 4$, the potassium conductance is negligible (interrupted line). The relatively high potassium conductance at the resting potential is obviously consistent with the relatively large initial current jump 'squaring off' obtained here and by others⁴ on membrane depolarization. It is also consistent with the initial current decay observed on membrane hyperpolarization by Chiu *et al.*⁶, and confirmed in the present work. The rate of this decay conforms with the projected values of τ_n at these potentials.

On the basis of the above, we suspect that the conclusion of Chiu *et al.* that (in the rabbit) this decaying current is capacitive (I_C) may be wrong. Moreover, because $I_C = C_M [dV_M/dt]$ (ref. 12), as $C_M \neq 0$ one must conclude that in their experiment $dV_M/dt \neq 0$, that is, the membrane was not fully voltage clamped throughout the first 0.5–1 ms.

We cannot attribute the almost completely square currents recorded by Chiu⁶ in the rabbit, and Brismar⁴ in the rat, to the shifted n_{∞} curve alone. However, this shift together with a somewhat depolarized membrane, as suggested by Chiu *et al.* themselves, may explain the results. Within the above framework, we may interpret the appearance of potassium currents following the rather drastic chemical and osmotic treatments in the rabbit⁷ as being due to a shift in the relative holding potential and perhaps also to a modification of the slow potassium activation process.

To rule out the possibility that in our experiments the myelin was lifted from the axolemma at the internode, we measured the nodal electrical capacity using the triangular wave voltage-clamp method¹². The nodal capacitance was found to be about 2.7 pF, compatible with that of the frog node¹³. The capacity did not change by more than 10% during the V_H changes which

induced the potassium conductance changes. These small C_M changes in the rat are definitely incompatible with the apparent 10-fold C_M change reported in the rabbit by Chiu *et al.*⁷ on the basis of the transient inward current obtained during membrane hyperpolarization.

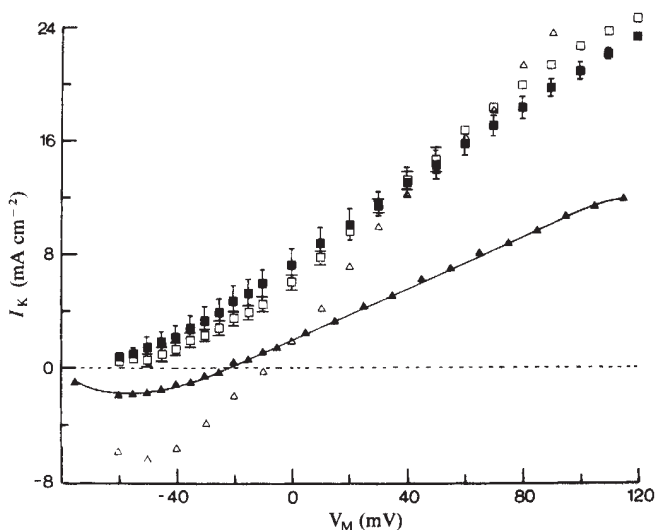
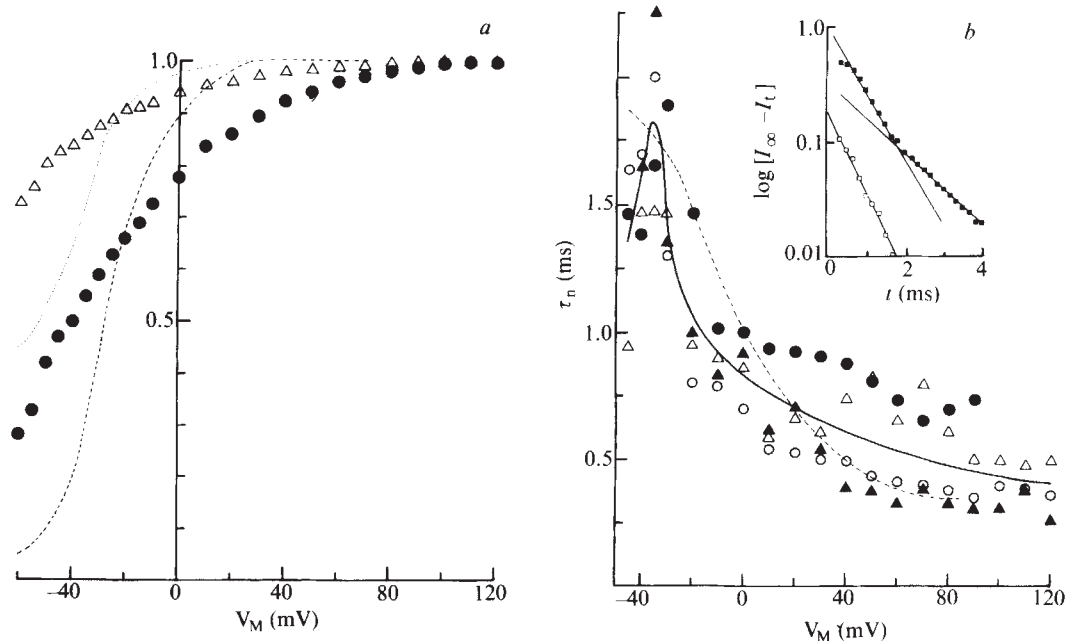


Fig. 2 Potassium current-voltage relationships measured from frog and rat nodes by means of the same voltage clamp. All currents corrected for leakage. Temperature 20 °C. $\blacksquare$, Potassium currents from rat node. Each symbol represents an average current value from six fibres $\pm$ s.d. To eliminate scatter of data due to 'g' effect (through, for example, variance in the number of channels) all currents of each fibre, were first normalized by multiplying each current value by the ratio of the current value at $V = 120$ mV to the average current value for all fibres at this potential. $\square$, Potassium currents from the frog node (*Rana ridibunda*). Each symbol represents an average current value from four fibres $\pm$ s.d. Current normalization was carried out as above. $\blacktriangle$, Potassium currents measured from a rat node when the external Ringer solution was replaced by an isotonic KCl solution. $\triangle$, Potassium currents from a frog (*Rana esculenta*) when the external potassium concentration was raised from 2.5 to 80 mM.

Fig. 3 Potassium conductance parameters in the rat node. Temperature 20 °C. *a*, The normalized steady-state potassium conductance (fraction of open channels). ●, n_{∞} values calculated from equation (1) for $x = 4$. △, n_{∞} values calculated from equation (1) for $x = 1$. Interrupted line: normalized conductance or fraction of open channels in frogs ($x = 4$), after Hille¹⁴. Note that close to the resting potential, at $V = -60$ mV, the relative conductance of the rat is at least five times larger than that of the frog. *b*, The potassium conductance time constants, τ_n , versus membrane potential. The symbols represent four rat fibres. Solid line, the average τ_n . Interrupted line, τ_n values in frog taken after Hille¹⁴.



Inset: the $\log [I_{\infty} - I_t]$ versus time (t) relationships for potassium currents at $V_H = -80$ mV (filled symbols) and $V_H = -71$ mV (open symbols). Note the double exponential at $V_H = -80$ mV, the disappearance of the second exponential when $V_H = -71$ mV and the similarity between the first exponential at -80 mV and the single exponential at -71 mV. Note also in Fig. 1*b* that beyond 2 ms the current traces for $V_H = -71$ mV become completely flat so that we could not continue the corresponding trace beyond this time.

Beyond the initial delay, the potassium currents show double exponential kinetics when plotted on a semilogarithmic scale (Fig. 3*b* inset). From the slopes of the initial exponential relationships we computed the potassium conductance time constant, τ_n . Figure 3*b* shows the voltage dependence of τ_n from four fibres. The shape of the curve and the absolute τ_n values are similar to those previously measured in frog¹⁴ (broken line) and in rabbit 'internode'⁶.

Thus, when compared with amphibian fibres, the delayed channels in the rat are characterized by the following features: (1) The n_{∞} - V_M curve is shifted towards more negative potentials, that is, at negative membrane potentials the n_{∞} values are much higher than the corresponding values in the frog and squid. This fact is responsible for the much larger conductances near the resting potential and as a consequence a large initial outward current step on depolarization. Many of the special properties of the potassium system in the rat, such as the current decay on membrane hyperpolarization, are probably due to this fact. (2) The values and voltage dependence of the initial time constant of the change in potassium conductance, τ_n , are very similar to the corresponding parameters in frog. This parameter is not affected by depolarizing V_H . (3) The initial increase in I_K , obtained on membrane depolarization, is followed by a smaller and slower increase or activation. The time constant of this slower activation process is about three times larger than the initial ' τ_n ' value. (4) Prolonged small membrane depolarizations (5–10 mV) attenuate steady-state I_K by 10–15% mainly by reducing the amplitude of the slow activation to virtually zero. The time constant of the development of this inhibition is >20 ms. Prolonged membrane hyperpolarizations increase steady-state I_K by 10–20%.

On the basis of the above findings, we believe that the excitability properties (threshold, action potential, refractoriness) of mammalian fibres in normal conditions can be analysed and reconstructed on the basis of models, similar in principle but modified in some details, with regard to the potassium system, to those developed for the frog and squid.

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Prolactin inhibits oestrogen synthesis in the ovary

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In 20% of cases of secondary amenorrhoea, hyperprolactinaemia prevents ovulation by impairing normal follicular development, but little is known of the biochemical basis for this effect¹. Bromocriptine can restore follicular growth and ovulation² by inhibiting the release of prolactin from the pituitary. The suckling stimulus causes an increase in prolactin levels³, and ovarian follicles fail to develop fully, thus inducing an anovulatory state throughout lactation in many mammals. We report here experiments with cultured granulosa cells which suggest that this contraceptive action of prolactin is due to its ability to interfere with the action of follicle-stimulating hormone (FSH) on the synthesis of oestrogen.

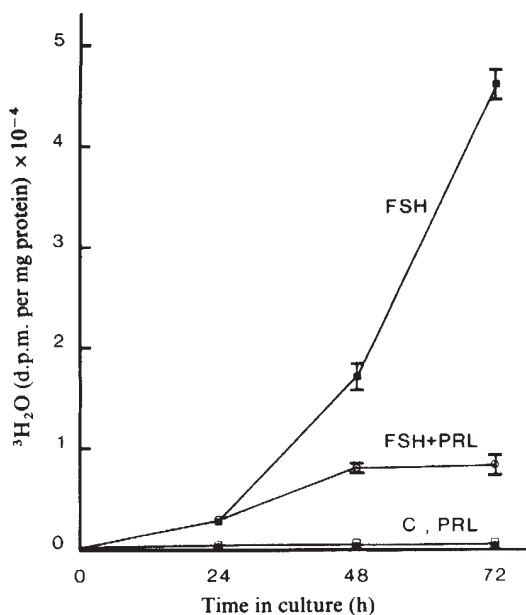


Fig. 1 Effect of FSH and prolactin on the aromatase activity of ovarian granulosa cells, assessed by the release of $^3\text{H}_2\text{O}$ from $[\beta\text{-}^3\text{H}]\text{testosterone}$. Granulosa cells were obtained from 25-day-old rats which had been treated daily for 3 days with 1 mg diethylstilboestrol in sesame oil. The cells were plated in Falcon 24-well tissue culture plates so that each well contained 100 μg protein and 0.5 ml modified Eagle's minimum essential medium, and were maintained at 37°C in a water-saturated atmosphere of 95% air and 5% CO_2 . Cells were treated with 300 ng NIH-FSH-S13 ml^{-1} and/or 1 μg NIH-P-S-7 ovine prolactin ml^{-1} 2 h after plating; the medium was changed after 24 and 48 h and appropriate treatments repeated. At 24, 48 and 72 h, 0.1 μCi $[\beta\text{-}^3\text{H}]\text{testosterone}$, containing unlabelled testosterone to give a final concentration of 0.25 μM , was added to four replicate cultures from each treatment group (C, controls; PRL, prolactin-treated; FSH, FSH-treated; FSH+PRL, FSH and prolactin-treated). The cells were incubated for 2 h at 37°C and the reaction stopped by placing the plates on ice. The medium was transferred to tubes and Norit A-activated charcoal (25 mg in 100 μl H_2O) was added. Incubations with charcoal for 2 h at 0°C completely adsorbed steroids without influencing the recovery of tritiated water. Steroids adsorbed to charcoal were removed by centrifuging at 4,000 r.p.m. and 4°C for 30 min. An aliquot (0.5 ml) of the supernatant was counted in 5 ml Aquasol.

The aromatization of the androgens, androstenedione and testosterone, to oestrogens in the ovarian granulosa cells, under the influence of FSH, involves a series of closely integrated reactions catalysed by an enzyme complex referred to as 'aromatase'. To assess the aromatase activity of granulosa cells after various hormone treatments, the stereospecific release of $^3\text{H}_2\text{O}$ from $[\beta\text{-}^3\text{H}]\text{testosterone}$ was measured—this assay has been validated as a specific measure of aromatization in granulosa cells⁵. Treatment of the cells with FSH shortly after plating in culture resulted in elevated aromatase activity after 24 h of treatment and a further increase after 48 and 72 h of treatment (Fig. 1). This effect of FSH confirmed earlier indications that it was involved in activating the aromatase system in immature granulosa cells, an important step leading to the formation of the preovulatory follicle⁶.

To investigate whether prolactin can influence this effect of FSH, prolactin was added alone, or together with FSH, to cultured cells. The amount of $^3\text{H}_2\text{O}$ released by the cell monolayers during a 2-h incubation with $[\beta\text{-}^3\text{H}]\text{testosterone}$ was measured after 24, 48 and 72 h of treatment. Prolactin did not affect FSH-induced aromatase activity for the first 24 h of treatment, but between 24 and 48 h it inhibited the effect of FSH; this suppressed level of activity was also observed after 72 h (Fig. 1). In other experiments, concentrations of prolactin

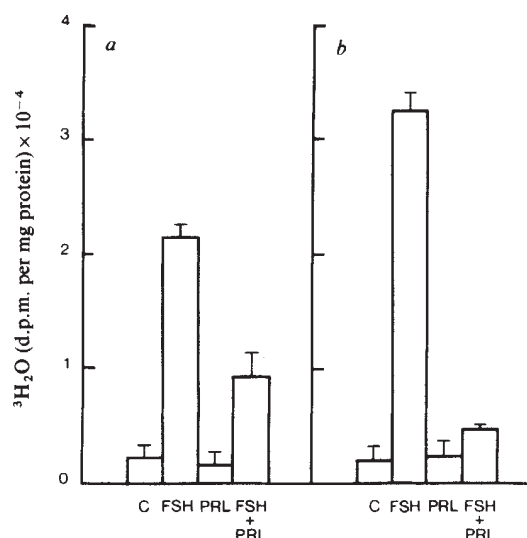


Fig. 2 Effects of FSH and prolactin on the aromatase activity of sonicates of ovarian granulosa cells using an optimal cell-free assay. Granulosa cells were prepared from diethylstilboestrol-primed immature rats and were cultured in 35×15 mm culture dishes in 1 ml Eagle's minimum essential medium for 48 (a) or 72 (b) h in the presence or absence of 300 ng NIH-FSH-S13 ml and/or 1 μg NIH-P-S-7 ovine prolactin ml^{-1} . Medium was changed and treatments repeated after each 24-h culture period. At 48 and 72 h, cultures from each treatment group were washed, cells were scraped from the plates and transferred to tubes in 20 mM phosphate buffer, pH 7.4. The cell suspensions were sonicated on ice and 0.2-ml aliquots of the sonicates dispensed into assay tubes containing the following reagents: 0.1 ml of 20 mM phosphate buffer; 0.1 ml of buffer containing MgCl_2 and dithiothreitol to give a final concentration of 20 mM and 5 mM respectively; 50 μl NADPH generator (final concentration of NADP, 0.5 mM; glucose-6-phosphate, 20 mM; and glucose-6-phosphate dehydrogenase, 20 U ml^{-1}); and 0.05 ml testosterone solution containing 0.4 μCi $[\beta\text{-}^3\text{H}]\text{testosterone}$ and unlabelled testosterone to give a final concentration of 0.25 μM . Incubations with gentle shaking were for 1 h at 37°C in an atmosphere of 95% O_2 and 5% CO_2 . The reaction was stopped by cooling tubes in ice-water and adding Norit A-activated charcoal (25 mg in 0.1 ml H_2O) to adsorb the steroids. Aliquots of the supernatant were counted in 5 ml Aquasol. Data are the means $\pm$ s.e.m. ($n = 3$).

from 10 to 100 ng ml^{-1} also inhibited the FSH-induced response. These concentrations are within the physiological range found in human follicular fluid⁷. No effects of prolactin alone were detectable during 72 h of culture, but this was not surprising as undifferentiated granulosa cells from immature animals do not contain specific prolactin binding sites⁸. Wang *et al.*⁹ showed that FSH stimulated the appearance of specific prolactin receptor sites on granulosa cells *in vitro* which may explain the 24-h lag before the effects of prolactin on FSH-induced aromatase activity were apparent.

To eliminate the possibility that prolactin influences aromatase activity by restricting the permeability of substrate or the availability of co-factors to the enzyme system, cells in culture were treated with hormones for various periods, after which cell sonicates were prepared and aromatase activity measured in the cell-free system in optimal conditions. As before⁵, pretreatment of granulosa cells in culture with FSH increased aromatase activity in the cell-free preparations (Fig. 2). Prolactin effectively reduced the amount of active aromatase induced by FSH during the first 48 h of culture and this effect increased after 72 h of treatment. Incubation of cell sonicates in optimal conditions showed that FSH and prolactin influenced aromatase activity in cultured granulosa cells by mechanisms which were independent of effects on the availability of substrate and

co-factor. FSH acts on granulosa cells to increase the amount of active aromatase, and prolactin seems to interfere with the ability of FSH to bring about this effect.

In addition to inhibiting FSH-induced oestrogen biosynthesis in rat granulosa cells, prolactin ($50\text{--}1,000\text{ ng ml}^{-1}$) affects steroidogenesis by stimulating progesterone synthesis⁹; the mechanism of two such different responses is intriguing. However, in human granulosa cells, high levels of prolactin seem to inhibit progesterone production¹⁰, and divergent effects have been demonstrated in porcine granulosa cells depending on the degree of differentiation¹¹.

When prolactin levels are high due to physiological (for example, lactation) or pathological (for example, hyperprolactinaemia) conditions, the inhibition of oestrogen biosynthesis and the concomitant changes in progesterone biosynthesis may establish a microenvironment in which the follicles can no longer develop normally and ovulation is suppressed¹.

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α -Lactalbumin is not a marker of human hormone-dependent breast cancer

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It has been proposed that milk protein gene expression in human breast carcinomas may indicate a functional oestrogen receptor mechanism, and may therefore be diagnostic of tumours responsive to endocrine therapy¹. Unfortunately this has not been proved, largely because of inconsistencies in the immunoassay procedures used to identify milk proteins, in particular α -lactalbumin, in tissue extracts or serum^{1–12}. Alternative procedures include the identification of milk protein mRNA by cell-free protein synthesis¹³, and the identification of milk protein RNA transcripts by hybridization to a sequence-specific probe. Here we describe experiments using α -lactalbumin cDNA probes, purified using recombinant plasmids¹⁴, which demonstrate that although polyadenylated and non-polyadenylated α -lactalbumin transcripts are present in normal human mammary tissue during pregnancy and lactation, α -lactalbumin transcripts are not detectable in the human tumour tissues studied. These experiments do however show that a peptide, which shares antigenic determinants with human α -lactalbumin is present in some breast tumour tissues.

Human and guinea pig α -lactalbumin cDNA hybridization probes were used in RNA excess hybridization studies to compare the relative amounts of human α -lactalbumin RNA transcripts in different RNA preparations. Guinea pig α -lactalbumin cDNA, which fortuitously has extensive sequence homology with the human α -lactalbumin cDNA¹⁴, was also used as only limited amounts of human milk protein mRNA were available for production of cDNA probes. As can be seen in Fig. 1, >60% of guinea pig α -lactalbumin cDNA was protected from S₁ nuclease digestion when hybridized with poly(A)-containing RNA from lactating human mammary tissue 15 days post partum¹³. Furthermore, the kinetics of hybridization were identical to those of the homologous hybridization using human α -lactalbumin cDNA. Analysis of poly(A)-containing RNA

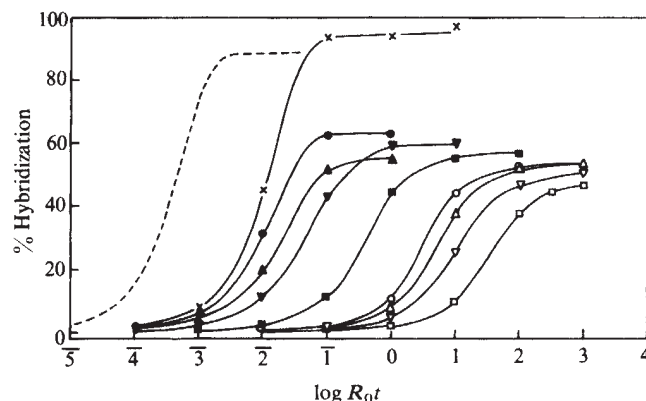


Fig. 1 Abundance of human α -lactalbumin transcripts in polyadenylated and non-polyadenylated RNA fractions isolated from normal mammary tissues of lactating and pregnant patients. Poly(A)-containing RNA was isolated from frozen human mammary tissue¹³ and hybridized either to human or guinea pig α -lactalbumin-specific cDNA as described elsewhere¹⁴. Non-polyadenylated RNA was defined as that which did not bind to oligo (dT)-cellulose at a salt concentration of 100 mM (ref. 15). The volume of individual hybridization assays (up to 100 μ l) and the RNA concentration (up to 5 mg ml⁻¹) were increased where appropriate to ensure an adequate RNA excess. Human α -lactalbumin cDNA versus poly(A)-containing RNA from 15-day post partum lactating human mammary tissue (x); guinea pig α -lactalbumin cDNA versus poly(A)-containing RNA from 15-d (●), 4-d (▲) and 330-d (▼) post partum mammary tissues, and normal breast tissue from a patient 32 weeks into pregnancy (■); guinea pig α -lactalbumin cDNA versus non-polyadenylated RNA from 15-d (○), 4-d (△) and 330-d (▽) post partum and 32-week pregnant (□) mammary tissue. The dashed line represents the homologous hybridization kinetics of highly purified rabbit globin mRNA standard¹⁴.

populations isolated from normal lactating mammary tissue 4 and 330 days post partum, and normal mammary tissue 32 weeks into pregnancy, using the guinea pig cDNA probe, demonstrated the presence of α -lactalbumin mRNA in each preparation but at different concentrations (Fig. 1). Compared with the 15-day post partum population, α -lactalbumin transcripts were present at 1.5-fold, 4-fold and 30-fold lower concentrations in the 4-day post partum, 330-day post partum and pregnant tissues, respectively. Analysis of the non-polyadenylated RNA from each tissue (RNA that did not bind to oligo (dT)-cellulose and contained polyadenylic acid tracts <35 nucleotides long¹⁵) showed that α -lactalbumin transcripts were present in all the preparations examined, again at different relative concentrations (Fig. 1). The overall analysis of normal human mammary tissue demonstrates that in common with the rat^{16,17}, mouse^{18,19} and guinea pig²⁰, α -lactalbumin gene

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expression occurs in the mammary gland before parturition as well as throughout lactation. In common with many mRNA species including other milk protein mRNAs, human α -lactalbumin mRNA is distributed between the polyadenylated and non-polyadenylated RNA populations¹⁵.

Similar examination of polyadenylated and non-polyadenylated RNA populations isolated from human mammary tumours produced unexpected results. Poly(A)-containing RNA was isolated from 12 breast tumours (including adenocarcinomas, infiltrating duct carcinomas and a benign tumour): six were examined using a human cDNA probe, one of which, designated T2, an invasive duct carcinoma, had previously been shown to synthesize α -lactalbumin as determined by a combination of mRNA-directed cell-free protein synthesis, antibody precipitation and SDS-polyacrylamide gel electrophoresis¹³. However, hybridization studies (Fig. 2a) showed that none contained α -lactalbumin transcripts as there was only limited hybridization when compared with controls (chick oviduct and human placental poly(A)-containing RNA). The remaining six, which were analysed using the guinea pig cDNA probe, also contained no α -lactalbumin transcripts (Fig. 2b), even though three of these tumours had been independently shown by radioimmunoassay to contain significant amounts of α -lactalbumin (1,029, 716 and 1,554 pg per mg protein). Two of those containing α -lactalbumin as determined by radioimmunoassay also had elevated levels of oestrogen and progesterone receptors. Analysis of the non-polyadenylated RNA

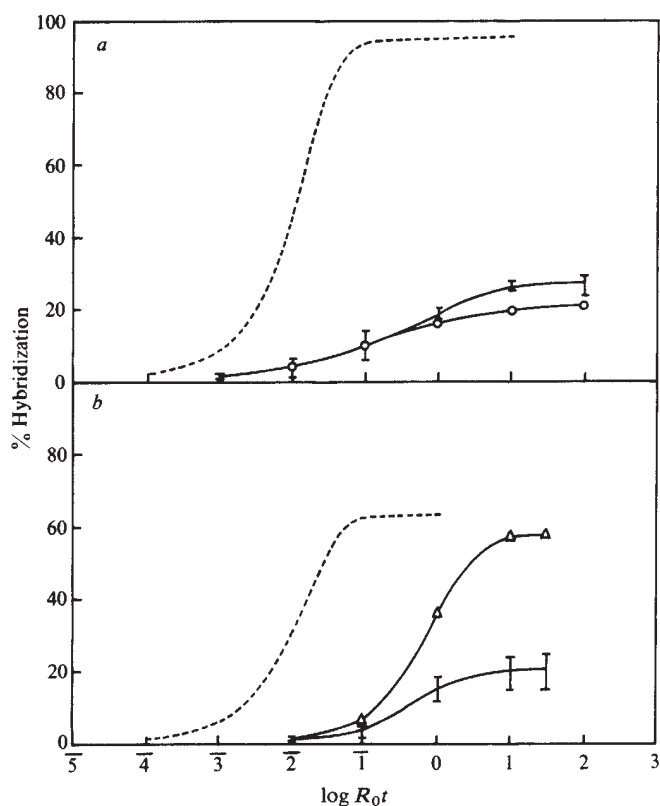


Fig. 2 Hybridization of poly(A)-containing RNA from human breast tumours to α -lactalbumin cDNA. Poly(A)-containing RNA was isolated from 12 frozen breast tumours¹³: six of these were challenged with (a), human α -lactalbumin cDNA and six with guinea pig α -lactalbumin cDNA (b). In each case the range of values obtained is indicated by error bars. Dotted lines represent the hybridization of poly(A)-containing RNA from 15-d post-partum lactating breast tissue to human (a) and guinea pig (b) α -lactalbumin cDNAs. Negative and positive controls were: (○), human α -lactalbumin cDNA versus human poly(A)-containing placental RNA; Δ , guinea pig α -lactalbumin cDNA versus a mixture of poly(A)-containing RNA from tumour and lactating tissue (99:1), respectively.

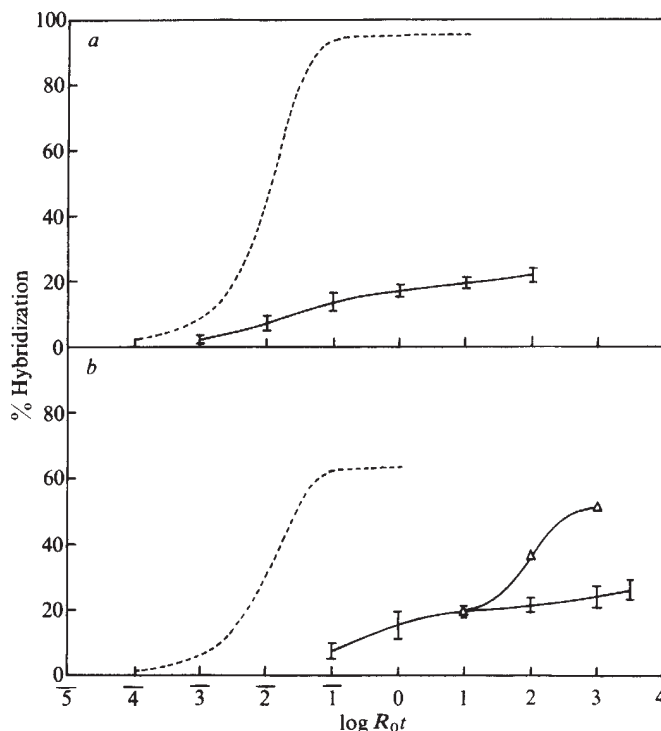


Fig. 3 Hybridization of poly(A)-containing and total cellular RNA from established tumour cell lines of human mammary origin, to α -lactalbumin cDNA. Twenty 9-cm-diameter dishes of semiconfluent cells were washed briefly with phosphate-buffered saline then lysed *in situ* with 10 mM Tris-HCl, pH 7.6, containing 1 mM EDTA, 2% (w/v) SDS, 0.5 mg ml⁻¹ proteinase K (1 ml per plate). The lysates were then processed as described for frozen tissue¹³. a, Human α -lactalbumin cDNA versus poly(A)-containing RNA from MCF-7 cells (both early and late passages, with and without added oestrogen²⁵), Hs578T cells and T-47D cells; error bars indicate range of values obtained. b, Guinea pig α -lactalbumin cDNA versus total cellular RNA from the above cell lines. Dotted lines represent the hybridization of poly(A)-containing RNA from 15-d post-partum lactating breast tissue to human (a) and guinea pig (b) α -lactalbumin cDNAs; Δ , guinea pig α -lactalbumin cDNA versus a mixture of MCF-7 total cellular RNA and polyadenylated RNA from 15-d post-partum lactating tissue (10,000:1).

from eight of the twelve tumours studied, including all those supposedly synthesizing α -lactalbumin, also proved to be negative (results not shown). As a positive control, poly(A)-containing RNA from 15-day lactating human tissue¹³ was mixed with a 100-fold excess of poly(A)-containing RNA from tumour tissue, then hybridized to guinea pig α -lactalbumin cDNA. This resulted in maximum hybridization (Fig. 2b) with the expected shift of hybridization kinetics (two logarithmic decades). It is unlikely that the absence of α -lactalbumin transcripts in tumour RNA preparations reflects degradation during extraction, as we estimated the mean size of the poly(A)-containing RNA to be 1,250 nucleotides in denaturing conditions, while cDNA prepared from the same RNA populations was of the same size, as determined by agarose gel electrophoresis in denaturing conditions.

We also analysed human cell lines of mammary origin: Hs578T²¹, T-47D²² and MCF-7²³ cell lines, all of which have been reported to synthesize either casein or α -lactalbumin²⁴. We found no evidence for the presence of α -lactalbumin transcripts, whether human α -lactalbumin cDNA was hybridized to poly(A)-containing RNA preparations (Fig. 3a) or whether guinea pig α -lactalbumin cDNA was hybridized to total cellular RNA (Fig. 3b). In the case of the MCF-7 cell line, RNA was analysed from early(15)- and late(275)-passage cells, and from cells stimulated with oestrogen²⁵, with negative results. As a

positive control, poly(A)-containing RNA from 15-day post partum lactating human mammary tissue was mixed with a 10,000-fold excess of MCF-7 total cellular RNA. This produced the expected degree of hybridization and shift in kinetics (Fig. 3b), thus confirming the specificity of the assay.

Overall it is apparent that α -lactalbumin gene expression does not occur to a measurable extent in any of the tumours or cell lines examined, using hybridization procedures which can detect as little as one polyadenylated, or <10 non-polyadenylated RNA transcripts per cell. The absence of α -lactalbumin RNA transcripts in the cell lines was not surprising, because although preliminary R_{ot} analysis and Southern blotting of cell-line and human placental restricted DNA demonstrated that the genes were present, we had been unable to demonstrate the presence of α -lactalbumin mRNA by cell-free protein synthesis (L.H. and R.K.C., unpublished data). However, the absence of α -lactalbumin transcripts from tumour tissue was unexpected, particularly as we have previously reported that a peptide with the expected electrophoretic mobility of pre- α -lactalbumin¹³ was synthesized in a cell-free protein synthesizing system directed by a tumour mRNA preparation, and that this peptide could be preferentially precipitated by α -lactalbumin antisera. This particular observation has proved reproducible using the same tumour (T2) mRNA and our own or an alternative source of α -lactalbumin antiserum. Because no α -lactalbumin mRNA was present in this tumour mRNA preparation, we conclude that both α -lactalbumin antisera preparations were reacting with a peptide which not only shared antigenic determinants with α -lactalbumin, but was also of the same apparent molecular weight as pre- α -lactalbumin.

Although we have not established the precise nature of the peptide(s) precipitated by antiserum to human α -lactalbumin, we consider that the close correlation between ' α -lactalbumin' and oestrogen receptors, reported to indicate a functional oestrogen receptor mechanism¹, may still prove useful once this peptide has been identified. Consequently we are screening our library of human mammary cDNA plasmids for those plasmids containing sequences that are abundant in tumour mRNA populations, several recombinants are being studied. Thus although the results are negative from a clinical point of view, we have demonstrated that sequence-specific cDNA hybridization probes generated using recombinant DNA techniques may soon be important as diagnostic tools in medicine. To conclude, using highly sensitive and specific hybridization techniques, we found no evidence to support the concept that α -lactalbumin will prove to be a general marker for human hormone-dependent breast cancer.

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Enhanced frequency of chromosome aberrations in lymphocytes of male compared with female muntjacs after X-ray irradiation *in vitro*

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Recently, there has been considerable interest in the study of radiation-induced dicentric and other chromosome aberrations in phytohaemagglutinin (PHA)-stimulated blood lymphocytes of various mammals, including man, with the aim of establishing a proper biological dosimetry for the assessment of genetic radiation hazards in human. These studies have revealed that the radiosensitivity of lymphocytes differs between species and even between individuals of the same species¹⁻⁵, but the cause(s) of these variations still remains unclear. The life-shortening response to whole-body X-ray irradiation is influenced by both age and sex of the individuals⁶⁻⁹, but although age has been shown to have effects on chromosome aberration yields^{4,10-12} *in vitro*, the influence of sex on the sensitivity of lymphocyte chromosomes to radiation-induced damage has not been reported. It is generally thought that sex does not influence the aberration yield within the limitation of measurements used in such studies. However, we report here a study of X-ray induced chromosome aberration in lymphocytes of Indian muntjac, in which the yield of dicentric was found to be consistently greater in male than in female lymphocytes.

We prepared lymphocyte cultures from healthy, young adult muntjacs (*Muntiacus muntjac*) selected from a small breeding colony which we maintained. The specimens were chosen so that we could do experiments on blood samples of three pairs of muntjacs of similar age and body weight. This barking deer has only six chromosomes in the female, with XX sex chromosomes, and seven in the male, with XY₂ sex chromosomes^{13,14}, and both the complements are distinguishable from each other by distinct constitution and morphology of the sex chromosomes (Fig. 1 a-d). This unique sex-based karyotypic dimorphism was used in a 'mixed blood culture' (MBC) method where whole blood drawn from males and females was mixed in equal proportion and then irradiated with various doses of X rays (110 kV, 4 mA, 1 mm Al and 100 rad min⁻¹ at 37°C) and cultured. This method ensures greater accuracy as it eliminates the possibility of errors inherent in irradiating and growing cultures separately for male and female individuals. The frequent metaphases for both sexes in MBCs, and the relative frequencies of first-, second- and third-division cells obtained at different times of fixation from male and female blood cultures set up separately, or after mixing, indicated that the normal progression of cell division had no obvious 'cross-reaction' in the MBCs. The results will be published in detail elsewhere.

A low concentration (0.1 µg ml⁻¹) of 5-bromodeoxyuridine (BUdR)¹⁵ was added at the start of culture and the chromosome preparations were stained using the Hoechst-sunlight-Giemsa technique^{16,17} to score aberrations only from the first-cycle

Table 1 Frequencies of dicentric and other aberrations in first-division metaphases of male and female muntjac lymphocytes in MBCs after X-ray irradiation

Muntjac pairs	Dose (rad)	Sex	Fixation time (h)	Cells scored	Normal cells	Dicentric ± fragments	Rings ± fragments	Breaks	Gaps	Deletions (TD + ID)
1 Male (Tomy) Female (Sindhu)	0	M	32	300	289	0	0	1	6	9
		F		300	293	0	0	0	3	10
	100	M	33	400	250	27*	2	11	40	89*
		F		400	278	14	7	9	43	61
	200	M	34	400	153	104†	11	17	43	210†
		F		400	199*	46	13	13	31	150
	300	M	35	300	62	128†	16	19	35	244
		F		300	87*	63	24	12	30	206
	400	M	36	200	15	117†	18	11	19	271
		F		200	29*	77	16	13	26	259
2 Male (Homy) Female (Bindu)	0	M	32	200	192	0	0	1	2	5
		F		200	194	0	0	1	1	4
	100	M	33	300	216	25*	6	8	11	34
		F		300	185	12	7	4	13	25
	200	M	34	200	101	39*	4	3	7	68
		F		200	117	22	4	4	9	55
	300	M	35	100	20	39*	5	12*	8	56
		F		100	24	27	6	4	9	79*
	0	M	32	200	195	0	0	0	2	3
		F		200	196	0	0	0	1	3
3 Male (Sony) Female (Rindu)	100	M	33	300	187	25*	3	18*	20*	77†
		F		300	282†	13	4	7	3	42
	200	M	34	200	85	41†	11	20	18†	64
		F		200	120†	19	4	14	2	64
	300	M	35	225	52	84†	19	12	19	177*
		F		225	85†	50	18	14	14	142
	400	M	36	100	10	56*	12	13	6	172†
		F		100	13	35	7	9	7	117

TD, terminal deletion; ID, interstitial deletion.

* Significant in χ^2 test, $P < 0.05$.† Significant in χ^2 test, $P < 0.01$.**Table 2** Frequencies of dicentric and other aberrations in first-division metaphases of male and female muntjac lymphocytes in MBCs fixed at 40 h after X-ray irradiation

	Dose (rad)	Sex	Fixation time (h)	Cells scored	Normal cells	Dicentric ± fragments	Rings ± fragments	Breaks	Gaps	Deletions (TD + ID)
Male (Tomy)	0	M	40	100	87	0	0	0	6	7
		F		100	90	0	0	0	9	2
Female (Sindhu)	100	M	41	280	169	27*	2	17	29	48
		F		280	179	12	8	10	38	55
	200	M	42	310	127	62†	14*	15	34	126
		F		310	144	33	4	20	24	127
	300	M	43	200	40	90†	15	5	15	148
		F		200	53	48	11	7	17	122
	400	M	44	150	11	84*	19	8	16	205
		F		150	21	57	17	8	17	172

TD, terminal deletion; ID, interstitial deletion.

* Significant in χ^2 test, $P < 0.05$.† Significant in χ^2 test, $P < 0.01$.**Table 3** Frequencies of dicentric and other aberrations in first-division metaphases from cultures set up separately for male and female muntjac blood after X-ray irradiation

Munjac pair	Dose (rad)	Sex	Fixation time (h)	Cells scored	Normal cells	Dicentric ± fragments	Rings ± fragments	Breaks	Gaps	Deletions (TD + ID)
Male (Tomy)	0	M	32	150	146	0	0	0	2	3
		F		150	147	0	0	0	2	2
Female (Sindhu)	100	M	33	150	99	13*	3	14*	13*	24
		F		150	117	4	1	4	3	22
	200	M	34	250	93	41*	15	15	12	112
		F		250	131*	24	8	18	18	94
	300	M	35	150	32	57*	11	22	11	119
		F		150	43	37	16	12	8	138
	400	M	36	150	12	73*	23	13*	14	172*
		F		150	15	49	27	4	12	135

TD, terminal deletion; ID, interstitial deletion.

* Significant in χ^2 test, $P < 0.05$.



Fig. 1 Metaphase chromosomes of male and female muntjacs. *a*, First-cycle and *b*, second-cycle metaphase preparations from female muntjac lymphocytes showing six chromosomes with XX. *c*, First-cycle and *d*, second-cycle metaphase preparations from male muntjac lymphocytes showing seven chromosomes with XY₁Y₂, a multiple sex chromosome system due to X-autosome translocation.

metaphases^{3,18-21}. For multiple sampling, fixation was done at 32 and 40 h to analyse aberrations. The frequencies of dicentric and other chromosome aberrations were scored from coded slides from 50–100 first-cycle male and female metaphases. The frequency of sister chromatid exchanges (SCEs) was scored from second-cycle metaphases and the experiments were repeated two to three times for each pair of muntjac.

The results obtained from the MBCs fixed at 32 h (Table 1) and 40 h (Table 2) as well as from the cultures of male and female lymphocytes set up separately (Table 3) indicated that the male lymphocytes were consistently more radiosensitive than female lymphocytes, as they showed a significantly higher (1.86 ± 0.25 , $P < 0.01$ or 0.05) frequency of dicentrics (Fig. 2). Although the frequency of deletions, which did not include fragments associated with dicentrics and rings, was quite high and at times showed significant variation between the two sexes, the frequency was not significantly different in most cases. With regard to symmetrical exchanges, there was sometimes a slight increase in the yield in females but the frequency was found to be statistically insignificant when compared with yields in males. The analysis was not done after banding of chromosomes, therefore their frequencies are not very precise the data on symmetrical translocations have not been shown. The yield of dicentrics in cultures set up separately for male and female was slightly lower than in the MBCs (Fig. 2) but the magnitude of difference in the yield of dicentrics between the two sexes still

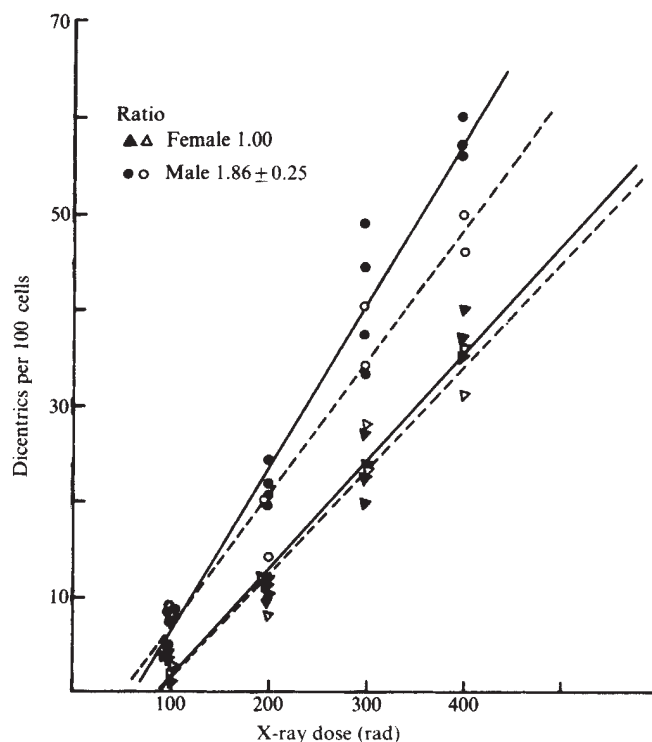


Fig. 2 Dose-response curves for dicentric in male and female muntjac lymphocytes grown in MBCs (●, male; ▲, female) and separately (○, ♂ male; △, ♀ female), after exposure to various doses of X rays. The curves are the least-squares regression lines of $y = a + bD$ ($r = 0.98$, $P < 0.001$).

remained the same. The frequency of other chromosome aberrations (Tables 1, 2, 3) however, did not show any significant difference between the two sexes, either in the MBCs or in the separate cultures. The frequency of SCEs was the same in male and female lymphocytes and was of the order of those occurring spontaneously, even after exposure to 400 rad (Table 4). This corroborates the suggestion that the mechanisms of production of SCEs and chromosome aberrations are different²⁰⁻²³.

Our previous study¹⁸ using the BUdR-harlequin technique on male muntjac lymphocytes fixed at 40 and 48 h after X-ray irradiation and initiation of cultures, and the present study of MBCs fixed at 32 and 40 h (Tables 1, 2) gave similar yields of dicentrics and rings in the first-cycle metaphases. This strongly suggests homogeneous sensitivity of muntjac lymphocytes to X-ray irradiation, which has also been reported for humans^{3,21}. Nevertheless, there are reports on human lymphocytes which suggest the presence of at least two subpopulations that differ in rate of progression of cell cycle and radiosensitivity^{20,24,25}. In view of the conflicting reports on human lymphocyte radiosensitivity, we scored dicentrics in lymphocytes of both sexes of muntjac only from the first wave of mitosis, mainly at 32 h, even though the yield of metaphases was very low at this hour, particularly in the case of male metaphases.

The significant difference observed in the frequency of X-ray induced dicentrics therefore indicates differential radiosensitivity of lymphocytes in the two sexes of muntjac. The low number of chromosomes in muntjac may suffer from 'distortion' in radiation-induced chromosome exchange analysis²⁶ but the slight difference in the chromosome number of males and females of muntjac is not expected to influence the yield of dicentrics in the two sexes. Thus these results reflect a real difference in radiosensitivity. Differential repair efficiency and/or other intrinsic factors may be responsible for this differential sensitivity. It is, however, interesting to note that in

Table 4 Sister chromatid exchange (SCE) frequency in control and irradiated cultures of male and female muntjac lymphocytes

X-ray dose (rad)	Sex	Total metaphases scored	Total SCEs scored	SCEs per cell $\pm$ s.e.
0	Male	50	363	7.26 ± 0.46
	Female	50	365	7.30 ± 0.42
100	Male	50	382	7.64 ± 0.72
	Female	50	448	8.96 ± 1.37
200	Male	50	341	6.82 ± 0.29
	Female	50	325	6.50 ± 0.59
400	Male	50	362	7.24 ± 0.39
	Female	50	382	7.64 ± 0.82

Down's syndrome, the presence of an extra chromosome 21 has been shown to cause a 50–100% increase in the incidence of radiation-induced exchange-type aberrations (dicentric and rings), whereas the frequencies of breaks and other intra-chromosomal changes are not affected^{10,11,27}. Although the situation is not parallel, we could assume that the presence of Y in male muntjac as an extra chromosome in the complement in some way accounts for the higher yield of dicentric. Experiments on other species with similar sex chromosome constitution, resulting in a difference in chromosome number between the two sexes, may elucidate this point.

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Altered ribosomal RNA genes in mitochondria from mammalian cells with chloramphenicol resistance

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Chloramphenicol resistance in mammalian cells is cytoplasmically inherited^{1,2}. In yeast, a similar phenotype is caused by mutations in the mitochondrial DNA (mtDNA), and sequencing of carefully constructed strains has identified nucleotide monosubstitutions in the 3' region of the large (21S) rRNA gene which correlate with the antibiotic resistance³. We have sequenced the corresponding section of mammalian mtDNA from chloramphenicol-resistant cell lines for comparison with the wild-type sequence. Differences between the sequences occur at positions similar to those altered in the yeast mutants, in a highly conserved region of the large (16S) rRNA gene.

The chloramphenicol-resistant cell lines investigated were 3T3CAP^R (ref. 4 and R. L. Siegel, unpublished data) derived from the mouse cell line 3T3, and the human line MC63 derived from HeLa B (ref. 5). Cytoplasm–cell fusion experiments have shown convincingly that the chloramphenicol resistance of these

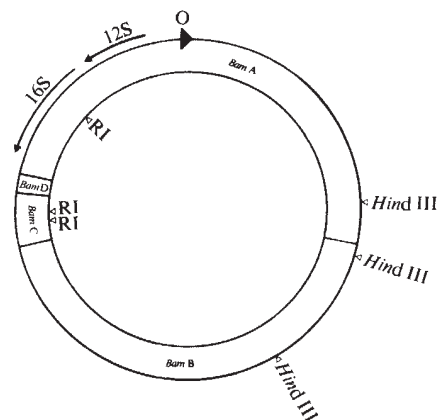


Fig. 1 The positions of *Bam*HI, *Eco*RI and *Hind*III restriction enzyme sites in mouse mtDNA²⁰; O denotes the H-strand replication origin, and the direction of transcription of the rRNA genes is marked²¹. The *Bam*HI-A fragment was purified by cloning in pBR322 (ref. 7), and the smaller *Bam*HI-*Eco*RI subfragment was isolated and digested with *Sau*3A. This was ligated into the double-stranded form (RFI) of a bacteriophage M13mp2 derivative adapted to accept *Bam*HI fragments^{9–11} (amber mutation in phage; G. Winter, unpublished results). Recombinant phage, which give colourless plaques on a lawn of *E. coli* JM101 (ref. 22), were grown up for screening as described in the text. The universal primer from pSP14 (ref. 23) was used for dideoxy sequencing¹⁴.

cell lines may be cytoplasmically transmitted to sensitive cells^{2,6}, although further genetic characterization has not been completed.

MtDNA from 3T3CAP^R cells was cloned as *Bam*HI restriction fragments using the plasmid pBR322 (Fig. 1 and ref. 7). Similar banks were also made using mtDNA from the parental cell line 3T3 and mouse liver. Plasmids containing the *Bam*HI-A fragment were prepared, allowing the purification of the smaller (1.3-kilobase) *Eco*RI-*Bam*HI subfragment from the plasmid insert. This contains the 3' end of the large (16S) rRNA gene (Fig. 1). The fragment was cut multiply with *Sau*3A for subcloning, using a bacteriophage M13mp2 derivative as vector^{8–10}.

Recombinant M13 clones from the mouse liver mtDNA bank were screened by partial sequencing reactions, using one dideoxynucleotide, ddT¹¹. Clones chosen for detailed sequence analysis were those containing restriction fragments having homology with the yeast 21S rRNA gene region in which nucleotide changes conferring chloramphenicol resistance are found³. As human and mouse mtDNA show high sequence homology in the rRNA genes¹², this screening was made easier by aligning the human 16S rRNA gene sequence¹³ with the yeast sequence. Comparison of the thymidine partial sequences of the mouse *Sau*3A fragments with the human sequence allowed the identification of several hybrid phage containing a 92-base pair fragment which spans the relevant region of the 16S rRNA gene. Two of these clones, containing this fragment in opposite orientations, were used to select clones from the 3T3 and 3T3CAP^R banks for dideoxy sequencing¹⁴. In this hybridization screening¹⁵, single-stranded DNA from clones containing the same restriction fragment as the reference clone, but in an opposite orientation, formed a hybrid with the reference clone DNA. These DNA hybrids have a lower mobility on agarose gels than the non-annealed single strands.

The MC63 mtDNA was digested with *Sau*3A and cloned directly into bacteriophage M13. Hybridization screening of 40 recombinant phages identified three homologous clones which were used for sequence determination.

Comparison of the mtDNA sequences obtained shows that the 92-base pair *Sau*3A fragments from 3T3CAP^R and 3T3 mtDNA differ by one nucleotide at position 30 (Fig. 2).

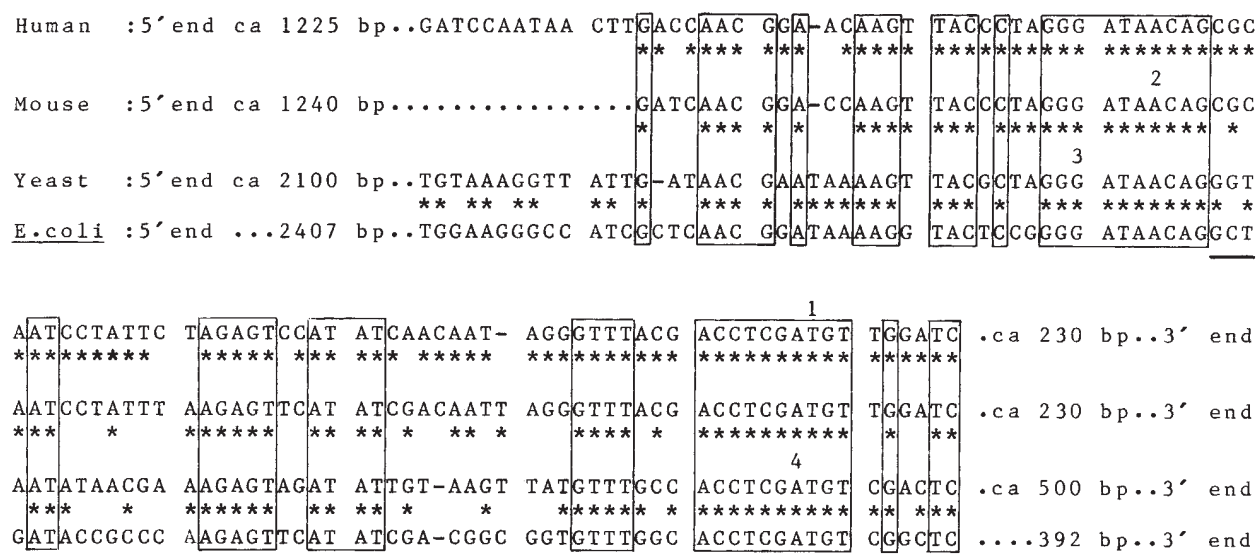


Fig. 2 Comparison of the DNA sequences in the 3' region of the large rRNA genes, showing positions of nucleotide changes in mtDNA from chloramphenicol-resistant cells. Sequences of the noncoding strand are given. The sequences compared are from human (placental) mtDNA¹³, mouse (3T3 or liver) mtDNA, yeast (*Saccharomyces cerevisiae* strain 1L9-8A/D122, with the C₃₂₃ site in its wild-type configuration) mtDNA³ and *E. coli* (*rrnB* gene)¹⁶. The sequences are aligned to show major blocks of homology, with gaps where appropriate. Asterisks show identity between adjacent sequences and boxed-in regions indicate sequences conserved between all four. Underlining indicates the position of the stem of a hairpin loop structure which is conserved between all four sequences. Nucleotide monosubstitutions in mtDNA sequences from chloramphenicol-resistant cells are indicated as follows: 1, MC63 (T to C); 2, 3T3CAP^R (A to T); 3, C₃₂₃ mutation in yeast (G to A)³; and 4, C₃₂₁ mutation in yeast (A to C)³.

Comparison with the yeast sequence data shows that this A-T transversion is only four nucleotides away from a homologous site at which a G-A transition in the *rib-1-B* locus causes the chloramphenicol resistance mutation C₃₂₃^R (ref. 3). This suggests that the sequence alteration is responsible for the antibiotic resistance phenotype of 3T3CAP^R although no functional genetic test is available. It is less likely that this mutation represents a silent polymorphism as the wild-type sequence compared is from the parental cell line. In any case, there are no differences between the 3T3 and mouse liver sequences (see Fig. 2) which suggests that polymorphism in the 3' region of the 16S rRNA gene is not high, and that the cloning steps used do not mutate the original nucleotide sequences.

The 104-base pair *Sau3A* fragment from MC63 mtDNA also shows one difference compared with the human placental sequence reported by Eperon *et al.*¹³ (Fig. 2). This monosubstitution, a T-C transition at position 96, is one nucleotide away from a homologous site in yeast mtDNA where an A-C transversion at the *rib-1-A* locus causes the chloramphenicol resistance mutation C₃₂₁^R (ref. 3).

The single nucleotide changes in the mtDNA from both the mouse and human mutant cell lines occur in short sequence blocks which are identical in the yeast mitochondrial 18S rRNA gene and the *Escherichia coli* 23S rRNA (*rrnB*) gene (Fig. 2 and refs 3, 16). Such conservation probably reflects functional constraints imposed on this region of the genome and suggests that a small genetic change, such as a base pair alteration, could have a considerable effect on the cell phenotype. Furthermore, this evolutionary stability makes it less likely that irrelevant polymorphisms are being detected.

Structural studies of the *E. coli* ribosome indicate that in the rRNA, the sequence shown in Fig. 2 occurs at the interface between the 30S and 50S subunits, the RNA being protected from kethoxal modification on subunit association¹⁷. This agrees well with the high degree of sequence conservation of this region through evolution compared with other parts of the large rRNA¹³. The nucleotides which are altered in chloramphenicol-resistant mutants are probably implicated in binding the 3' terminus of aminoacyl tRNA, because chloramphenicol blocks

this site¹⁸. A hairpin loop can be constructed between the mutation sites, and this feature is conserved despite local divergence of the primary sequences compared in Fig. 2 (ref. 19). If this structure occurs in the ribosome it would bring the putative chloramphenicol-binding sites together.

Further sequence analysis of mtDNA from other chloramphenicol-resistant cell lines should help to confirm the correlation suggested here. Chloramphenicol resistance is the only readily obtainable genetic marker in the mammalian cytoplasm, and a simple molecular basis for the mutation might help to explain why this is the case.

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MATTERS ARISING

Sexual dimorphism in early anthropoids

FLEAGLE *ET AL.*¹ provide an estimate of sexual dimorphism in extinct anthropoid primates based on a positive relationship between the degree of sexual dimorphism in body weight and the coefficient of variation (c.v.) of mandible depth in extant anthropoids. A cursory glance at Table 1 shows that their data on the degree of body weight dimorphism are incorrect. As a striking example, capuchin monkeys (*Cebus apella*) are supposedly more dimorphic than gorillas (*Gorilla gorilla*) which, as any visitor to the zoo knows, is simply not the case. Gorillas are far more dimorphic than capuchin monkeys.

A closer examination of the data reveals that 9 (those taken from our study on sexual dimorphism²) out of 13 species do not express a mean male weight/mean female weight ratio as intended by Fleagle *et al.*, but instead show a ratio between the cube roots of male and female weights. In our study we used the cube root of body weight to obtain a linear dimension comparable to a linear canine dimension. Using corrected data for body weight, I recalculated its correlation with the c.v. of mandible depth and obtained a correlation coefficient, $r = 0.597$, which is substantially lower than $r = 0.940$ as calculated by Fleagle *et al.* This leaves >60% of the variance unexplained, which in turn indicates that the coefficient of variation is a much poorer predictor of the degree of sexual dimorphism than they suggest. A solid quantitative method for estimating the degree of sexual dimorphism in fossils based on the variability within a species and independent of prior sex determination of individuals still eludes us.

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FLEAGLE *ET AL.* REPLY—Leutenegger correctly points out that we inadvertently mixed the body size data in Table 1 of our paper¹ and therefore overestimated the correlation coefficient. In rechecking our original data, it turns out that we incorrectly transcribed the c.v. of mandibular depth for *Cebus apella* as well; the correct value is 14.5. When the

correlation coefficient for the entire sample was recalculated, using the cubed roots of body weight as suggested by Leutenegger, the correct value for r was 0.755. Although this value is lower than that previously reported, it is still highly significant ($P < 0.01$) and demonstrates a positive correlation between c.v. of mandible depth and sexual dimorphism in body weight. This correction in no way affects our major points: that the coefficient of variation for mandible depth and the amount of canine dimorphism in the Fayum anthropoids are considerably higher than that found in any of the monogamous taxa.

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Enzyme heterozygosity and morphological variance

HANDFORD¹ recently reported a lack of association between enzyme heterozygosity and measures of morphological

variance in the Rufous-collared sparrow. He suggested that this negative case, which was in apparent contrast to the findings of myself² and Mitton³, could be explained by an inherent difference, with regard to this relationship, between homoiotherms and poikilotherms. Although Handford makes several statistical points worth consideration, he ignores the fundamental problem of statistical power in his analysis. Essentially, his data set is so small that unless we assume the putative relationship between heterozygosity and morphological variability to be extremely strong, the ability to reject the null hypothesis of equal variances will be very restricted.

If enzyme heterozygosity does exert some influence on morphological variability, our biological intuition suggests that the contribution of individual loci will be subtle. Examination of the data sets for the monarch butterfly² and the killifish³ clearly support this notion. For example, the ratio of homozygote to heterozygote variances in the monarch is only 1.17 for the entire set of loci. This does not diminish the evolutionary importance of such a relationship, but rather places realistic expectations on the magnitude of the effect. Furthermore, because we are comparing variances, the ability to make strong statistical inferences about such associations will be constrained without large sample sizes and many independent observations.

The statistical power for the variability measures and sample sizes reported by Handford² is difficult to quantify. Regardless of the drawbacks of the F -test, it can be used in a simple univariate case to illustrate the limitations of these sample sizes. For a normally distributed character, the homozygote/heterozygote F ratios associated with Handford's larger sample sizes which would be statistically significant ($P < 0.05$) are 1.8 (d.f. = 130, 22) and 1.5 (d.f. = 76, 49). These are unrealistically large ratios given the subtlety of the relationship expected and observed in other organisms. The problem of type II error does not disappear by using Handford's particular treatment or by applying the sign test. In addition, Handford uses multivariate measures of variability which further reduces the statistical power with the inclusion of each new character. Indeed, three of the five tests for matrix homogeneity in females cannot be carried out because the sample sizes are so small that there are insufficient degrees of freedom.

His cautionary statement that one nonsignificant result does not negate the relationship is true, but a nonsignificant result for a truly powerful data set would have permitted some strong inference to

be made. Obviously, the need is not for many more tests of weak statistical power, but rather for a few tests, each with sufficient power to distinguish between no relationship and one of small, yet realistic, effect.

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HANDFORD REPLIES—Of course, I agree with what I take to be Eanes' main point: that many more data are needed to clarify the relationship between heterozygosity and morphological variability. Indeed, my modest contribution¹ closed with an implied request that workers (and there must be many) in possession of data sets containing the necessary information (for many taxa and based on larger samples) do the necessary sums and communicate their results. In that note¹ I also made the obvious point that lack of a significant result does not demonstrate much on its own (even though nonsignificance is also evident from the sign test² used by Mitton³ in the interpretation of his results).

Regarding statistical considerations, Eanes is correct about necessary magnitudes of effects when using the *F* ratio. Comment has been made about the use of *F* ratios¹, and will not be repeated. However, I may compare my data with those of Mitton³. If his three localities are considered separately, his samples are comparable, both biologically and numerically, with mine¹. In all three samples, Mitton's data yield a result which is significant by the sign test; mine do not. The sign test is, of course, independent of the magnitude of the differences in variance and of the size of the sample used in computing the variances. Given that there are some *a priori* reasons for expecting that heterozygosity may be of different biological significance among homoiotherms and poikilotherms¹, I felt my results worth communicating.

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Nigral-derived muscimol circling—ipsiversive, contraversive or controversial?

REAVILL *ET AL.*'S¹ attempted reconciliation of the dispute between Waddington² and Martin and Haubrich³ raises some important issues. Briefly, the controversy centres on whether disinhibition of dopamine (DA) neurones in the substantia nigra zona compacta (SNC) contributes to the contraversive circling produced by injecting a γ -aminobutyric acid (GABA) agonist into one nigra, as this has been variously reported as being unaffected^{4–6}, attenuated^{7–11} or even exaggerated¹² by measures which reduce nigrostriatal dopaminergic activity. We, too, firmly believe that the precise location of injection of the drug within the nigra holds the key to these disparities. Thus when muscimol (40 ng) is microinjected discretely (in 0.2 μ l over 3 min) into the central region of the zonal reticulata (SNR) at all rostrocaudal levels, it evokes rapid contraversive circling which is significantly (though never completely) inhibited by pretreating the animals with haloperidol (0.1 mg per kg) or pimozide (0.25 mg per kg), intraperitoneally, or 6-hydroxydopamine (4 μ g into the SNC or medial forebrain bundle). That is, it is partially DA dependent (see refs 7–11). On the other hand, injecting the agonist into ventral or lateral aspects of the SNR, irrespective of rostrocaudal coordinates, again stimulates rats to move in contralateral circles, but in this case the rate of rotation is regularly slower and is unaffected by the above treatments. In other words it is DA independent (see refs 4–6). Consequently we believe it is the disposition of muscimol in a mediolateral or dorsoventral direction within the reticulata, and not along the rostrocaudal plane of the nigra as a whole as Reavill *et al.*¹ surmised, that determines whether the resultant circling behaviour involves dopaminergic activation.

The latter could occur by muscimol suppressing the activity of inhibitory interneurons, or of nigrofugal fibres that send collaterals to the SNC. Our suggestions are in keeping with the known topography¹³ and electrophysiology¹⁴ of this area of the nigra. The assertion that Martin and Haubrich could only have achieved striatal DA release and contraversive circling with muscimol from the caudal nigra is therefore unfounded. Furthermore, our arbitrary division of the SNR into two longitudinal bands from which different types of contraversive circling may be elicited should not be confused with the separate, weaker depressant action of muscimol on the compacta DA neurones directly¹⁴. This will give rise to ipsiversive asymmetry that is totally abolished by neuroleptics⁵. We would agree that this type of ipsiversive

behaviour is most readily evoked from the rostral SNC. In fact, we have never observed ipsilaterally directed movements with muscimol from the caudal SNC. Thus a behavioural distinction can be made between GABAergic drugs acting on the SNC (rostrally) or the SNR (caudally), but this does not account for the above-mentioned discrepancies.

To dismiss as 'redundant' the significance of striatal DA release in the mechanism of nigral GABA-mediated circling could be premature, as this presupposes that all such behavioural influences are necessarily channelled back through the SNR, which has yet to be ascertained. This might be true for rotational behaviour generated artificially from the SNR by an agonist as powerful as muscimol, even in 'thalamic' rats¹⁵, but we cannot help wondering if animals bearing chronic cerebral damage can validly be used to predict the importance of events that take place in the whole animal. The elegant experiments of Grace and Bunney¹⁴ favour the opposite view by showing that the firing rates of compacta DA cells are reciprocally related to the activity of non-dopaminergic GABA-receptive cells in the neighbouring SNR. The focal ejections of GABA which raised DA cell firing severalfold were made into the central region of the SNR, the area most densely innervated by striatonigral GABA terminals¹⁶. Cells here are exquisitely sensitive to iontophoretic GABA and it is from this region that we obtained contraversive turning to muscimol which was strongly dependent on dopaminergic mechanisms. There is every reason to suppose, therefore, that these GABA–DA interactions are far from inconsequential in normal physiological conditions.

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BOOK REVIEWS

Oil in the wheels of politics

Ian Smart

THOSE who have to catalogue or identify books would sometimes welcome a little more care in choosing titles. Certainly, no one should reach for this stimulating but strangely uncertain symposium in the belief that it will offer a synoptic view of the relationship between energy and security. On the supply side, coal, gas, nuclear power and "renewables" are mentioned in passing, but the emphasis is otherwise exclusively on oil. On the demand side, changes in oil and total energy consumption are duly noted, but the structure and evolution of conversion and end-use patterns are largely ignored. So are all questions of energy technology. As to security, it is understandable that most of the discussions and policy recommendations relate to the United States, albeit against the background of praiseworthy insistence that American isolation from world energy concerns is impossible. What is less acceptable is the absence of any serious reflection on what "security" really means in the energy context. Some of the groundwork is here, for instance in the occasional distinction between import dependence and supply vulnerability. In the end, however, the only definition of energy security, implicitly or by exclusion, is in the all too familiar form of immunity to a sudden disruption of flows or prices in the world oil market. The result is a book about short-term threats to oil trade, with particular reference to the United States. That is undeniably an important subject, but only a broader analysis could hope to determine its true significance.

Any volume made up of separate papers derived from a series of seminars and written by both senior luminaries and graduate students confronts its editors with problems of coherence and consistency. This is no exception. In minor matters — mis-spellings or misprints — the standard is sometimes deplorable. In more important respects, however, there is better evidence than usual of thematic continuity. Nor are the more junior contributors disgraced. Indeed, Fen Hampson on Canadian energy policy and, outstandingly, Kevin Middlebrook on US–Mexican energy relations make two of the most useful and polished contributions to the collection. Some of the problems nevertheless prove insuperable. It has to be said, for example,

Energy and Security. Edited by David A. Deese and Joseph S. Nye. Pp.489. ISBN 0-88410-640-3/0-6660-684-5. (Ballinger/Harper & Row: 1981.) \$14.50, £9.50.

that a book so narrowly focused on oil politics deserves a less superficial survey of politics in the Gulf: a chapter which might, moreover, have carried more weight if, like other sections, it had been reviewed since the outbreak of the Iraq–Iran war. In general, however, the quality of the individual contributions is sufficient, and sometimes exemplary. The intractable difficulty is rather one of reconciling what is said or, more often, implied in the different papers.

In some cases, the impression created is that apparent conflicts of interpretation have been recognized but have resisted resolution. In their summing-up of lessons for American policy, for instance, the editors (with Alvin Alm) strongly endorse the establishment and use of national oil stockpiles, but without referring to some hard questions about that policy raised earlier by Thomas Neff, in a strong paper on recent changes in the oil market. In other cases, the impression is that the participants have simply not addressed themselves to fundamental questions begged by the conjunction of their separate analyses. Two such questions stand out. One is about the relationship between governments and energy markets. And the other is about the relationship between immediate protection against oil-trade disruptions and the ultimate security of energy systems at large.

The contributors are especially uncertain about the role of government. In his opening chapter, Joseph Nye argues for more government involvement in external energy affairs, but less in domestic ones. Other authors echo that view, extolling the usefulness of market forces and casting sceptical glances at the ability of government to regulate or *a fortiori* to ration the flow of oil in an emergency, or even to shape the domestic energy economy in the longer term. Yet, time and again, recommendations for policy action — on stockpiling, pricing, taxation, revenue distribution or the promotion of alternative supply — imply a need for more, rather than less, governmental intervention. The domestic market must be

made free, so that it can transmit price signals efficiently to consumers, but the market must be managed, lest the signals it transmits, as in either the easy days of 1975–1978 or the panic of 1978–1979, reflect an inefficiently short perspective. (David Deese and Linda Miller, in discussing the West European dimension, even complicate the issue by suggesting that, in an emergency, available oil will gravitate towards "free market countries".) Nowhere is there convincing evidence that the contributors and editors have faced up to the obvious dilemma lurking in those shadows. The suspicion they leave is of having convinced themselves that government, in the abstract, must play a role of which American governments, in the real world, are incapable.

The second fundamental but unresolved issue is even more obvious. Several of the authors, led by William Hogan, apparently believe that short-term measures to prepare for disruptions of oil trade have been neglected in favour of efforts to limit oil imports or oil consumption in the longer term. In the particular case of the United States, given the fiasco of the Strategic Petroleum Reserve, it would be hard not to sympathize with them. What is lacking, however, is a considered sense of the opportunity cost of short-term oil emergency measures in relation to the longer-term management of fuel supply and of the energy economy as a whole. A country's relative security in regard to energy palpably depends on much more, in the final analysis, than the scale and pattern of its oil imports. Far larger social, political and institutional, as well as economic, questions are at stake. In this case, however, they remain unilluminated. Anyone who wants to think more deeply about the problems of coping with another Arab oil embargo or the effects on oil supply of another Middle Eastern war, especially in the United States, should read this book. But oil supply is not the only element in national, let alone global, energy security, and anyone who wants to learn about other elements of that now perennial issue will look here in vain.

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States of matter inside the planets

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Interiors of the Planets. By A.H. Cook. Pp.348. ISBN 0-521-23214-7. (Cambridge University Press: 1980.) £25, \$59.50.

Interiors of the Planets, a volume in the Cambridge Planetary Science series, is intended as a textbook for advanced students. The book reads rather more like an extended review article than a text, however; yet probably no other approach is possible in this rapidly-developing field.

A number of thorough, up-to-date accounts of important topics are scattered through the book. The initial discussion of the Earth and the derivation of interior models from seismic data and the Adams-Williamson equation is lucid, as is the subsequent coverage of heat sources in the Earth and effects on the temperature gradient. The strongest parts of the book are those which deal with measurement of gravitational field parameters and with solid-body planetary motions — the use of artificial satellites, fly-bys and laser retro-reflectors to determine gravitational field parameters and precession and libration constants is considered in some detail; and the first-order theory of figures, leading to the important Radau-Darwin approximation, is given in enough detail to make clear its physical significance.

There are two chapters on equations of state — separated by chapters on the Moon and terrestrial planets — a division which seems a little curious, since similar physical principles are involved. Nevertheless, the exposition of experimental techniques for measuring equations of state and phase transitions is a helpful guide to the intricate transformations which can occur in minerals at high pressure. The chapter on metallic and molecular hydrogen, which comes later, is also fairly complete, and gives the reader a good feeling for the convergence of very different methods for deriving pressure-density relations and other important thermodynamic parameters.

The chapter on the Moon is somewhat sketchy. Lunar seismology and the evidence for a lunar core from the moment of inertia are presented in useful detail, but an adequate discussion of important work on electromagnetic sounding of the lunar interior is lacking. The account of the formation of the lunar crust tends to be unclear — and many workers in this field would doubt the assertion that the crust may have accreted separately onto the Moon, from meteorites, after formation of the mantle.

The discussion of Mars, Venus and Mercury is likewise rather uneven. Moments of inertia are dealt with well, and the author succeeds in giving a clear, simple exposition of the relation between the moment of inertia and the relative effects of crust, polymorphic phase transitions

and core. On the other hand, the effects of tidal dissipation are ignored, and the classic work of Goldreich and collaborators on spin-orbit coupling in terrestrial planets is mentioned only obliquely. Although some attention is given to the higher-order gravity fields of the Earth, Moon and Mars, a geoid map of each is needed for clarity; such a map is given for the Moon only, but without definition of units.

Similarly, the chapter on the Jovian planets has some fairly major gaps. Most discussions of the composition of this group start with the mass-radius diagram for self-gravitating spheres at zero temperature. The existence of a pronounced maximum radius for any such object at about the Jovian radius is convincing evidence for the preponderance of hydrogen in Jupiter and Saturn. Without such a diagram, the reader must take this important result on trust. In

addition, the discussion of the heat balance of Jupiter and Saturn is misleading, and it is quite impossible for the solar wind or ultraviolet radiation to contribute in a significant way to the observed imbalance.

The author makes it clear that *Interiors of the Planets* is not a cosmogonically oriented book. His general theme is the use of established physical principles to draw general conclusions about the present interior state of planets. A synthesis of the derived interior structures of planets does require cosmogonical considerations, but we do not yet know enough to carry out such a synthesis.

The book will not suffice as a sole reference to planetary interiors for an interested student, but it will be a helpful addition to his library. □

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Logic in Galilean scholarship

A. Rupert Hall

Galileo and the Art of Reasoning: Rhetorical Foundations of Logic and Scientific Method. By Maurice A. Finocchiaro. Pp.478. Hbk ISBN 90-227-1094-5; pbk ISBN 90-227-1095-3. (Reidel: 1980.) Hbk Dfl.80, \$42; pbk Dfl.40, \$21.

THIS is, as befits the series in which it is published, very much a book by a philosopher for philosophers, a book about logic rather than about Galileo. A scientist, a historian or a general reader wishing to make an initial exploration of Galileo's style as a writer and a natural philosopher would do much better to sit down with an English version of Galileo's *Dialogues on the Two Chief Systems of the World* than to read this volume. Indeed, Professor Finocchiaro's study would be impossibly hard to follow for anyone who has neither familiarity with Galileo's book, to which it is directed, nor with contemporary scholarship in history and philosophy of science.

This is, in brief, a work of detailed and technical criticism, mostly of the logical structure of arguments offered either by Galileo himself or by would-be interpreters of Galileo, both historians and philosophers (and at least one great scientist — Einstein). Finocchiaro exercises singular skill and acuity in the close analysis of reasoning in order to test whether opinions and generalizations purportedly justified by it are valid or otherwise: most commonly he finds them otherwise. Thus he finds grounds for strong reservations about statements or views relating to

Galileo put forward by Alexandre Koyré, Paul Feyerabend, Maurice Clavelin, Dudley Shapere, William Shea, Stillman Drake and many others. To give one example of the elaboration involved: a quotation from a book by Koyré published 42 years ago occupies half a page, its dissection 14½ pages (admittedly of larger print). It is the fine structure of the Koyré passage that is faulted: "it would be unhistorical" the author concludes "to deny that the study of the history of science made great progress with Koyré: to turn the clock backwards is simply unthinkable".

It seems that his object is to make philosophical judgements upon the philosophical positions adopted by other writers on Galileo: Koyré's error lay in his being an apriorist rationalist; Feyerabend's in denying the rationality of Galileo's and modern science; Drake's in being an empiricist or perhaps totally aphilo-sophical, and so forth. This makes the road to a positive understanding of Galileo's reasoning long and hard. On the other hand, Finocchiaro does express plainly his sense of the merits of previous scholars, even though (in his view) all have made serious mistakes, and he has studied the *Dialogue* and the difficulty of translating it with exemplary care, providing very full analyses of both the content and the nature of the arguments used by Galileo.

There are, to me, paradoxes in Finocchiaro's book apart from the paradoxes of Feyerabend, the Zeno of out time. Why does he object to Koyré's writing that the *Dialogue* is "not a book of astronomy, nor even of physics. It is above

all a book of criticism, a work of polemic and struggle; it is at the same time a pedagogical work, and a philosophical work. . .? Why does he write of Galileo's allegedly "Platonic" hypothesis of the planets all being accelerated into their orbits by falling from a unique point in space that "the correspondence is amazingly close"? (It is not, as Newton pointed out long ago in his second letter to Richard Bentley, though Galileo seems to accept it as true.) Why is it profitable or illuminating to treat Galileo's *Dialogue* in case-study fashion as though it were a statement of contemporary science?

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It is fair to end with some (abbreviated) conclusions of the author's own:

The evidence from Galileo indicates that science is primarily a method rather than a set of abstract truths; that method involves not the fixed adherence to some formal universal rules, but rather the judicious balancing of such opposites as speculation and observation, quantitative analysis and qualitative considerations. . .; that in particular such judgment does not automatically exclude rhetorical persuasion or aesthetically expressed emotion; but that such exercise of judgment reduces ultimately to a matter of reasoning, namely the drawing of conclusions from premises and the formulation of reasons for claims.

Finocchiaro ends with the admonition that "in order to become more scientific, the study of reasoning needs a greater empirical orientation towards reasoning as it actually occurs in the world" to be found by employing a "broadly conceived historical approach" not shying away from "attempts at concrete theoretical systematizations". □

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Essential techniques in diazotrophy

John Postgate

Methods for Evaluating Biological Nitrogen Fixation. Edited by F.J. Bergersen. Pp.702. ISBN 0-471-27759-2. (Wiley: 1980.) £38, \$114.

To do justice to a book as important as this in a short review is a frustrating task. It is a compendium of modern methods of studying diazotrophy and, as most readers will know, it is largely new (or improved) techniques which have permitted the spectacular advances of the past few decades.

First, a brief survey of its authors and scope. Dalton describes techniques for the culture of nitrogen-fixing bacteria; Bergersen discusses direct nitrogen analyses (for example Kjeldahl, $^{15}\text{N}_2$); Turner and Gibson deal with indirect assays of diazotrophy (acetylene reduction, hydrogen evolution); Gibson copes alone with methods for handling legumes in a laboratory or glasshouse; Bond and Wheeler give details for examining actinorhizal symbioses.

More biochemically, Eady comprehensively surveys methods of extracting, purifying and studying nitrogenase; Appleby and Bergersen deal with leghaemoglobins; Farnden and Robertson attempt, with considerable success, the almost impossible task of deciding what enzymes are metabolically related to nitrogenase, and tell how to handle them — they give priority to plant enzymes. Schwinghamer and Dudman then make a somewhat discursive job of "methods for identifying strains of diazotroph", straying rather often from true practicality and regrettably concerning themselves almost exclusively with rhizobia. In contrast, Cannon's terse chapter on everything you need to know to study the genetics of diazotrophs is a masterly laboratory manual in itself.

Brockwell's wide experience informs his chapter on crop and pasture legumes, which takes the reader out into the field; a comparable authority pervades Thompson's chapter on legume inoculants. Dobereiner describes the relatively new if controversial area of diazotrophic associations involving grasses and grain crops — her account of the much-criticized excised root acetylene assay, as well as other techniques, is useful and balanced. Knowles's chapter on plant communities and soils extends coverage to natural ecosystems; finally, Stewart presents a synoptic view of the cyanobacteria and their associates, detailed and down to earth on the free-living types, less practical on the symbioses.

With such broad coverage some defects are inevitable and I must mention a few. Someone ought to have warned about the hazards of "ghosts", those bacteria which scavenge atmospheric fixed N (NH_3 or

NO_x) and so simulate diazotrophs, for they are still responsible for occasional false reports of diazotrophy. Bergersen should have mentioned the use of ammonia electrodes, and also warned researchers not to smoke, sweat or permit reagent ammonia in the room (or work near stables!) when doing Kjeldahl analyses. Thompson ought to have warned of the inherent error of Petroff-Hauser chambers (they over-estimate). Indeed, enumeration of diazotrophs can be tricky and it is inadequately discussed. Dobereiner considers the enumeration of some, but the topic ought surely to have been in Dalton's brief. Dalton also omitted the important problem of colonial multimorphism in putatively pure strains of rhizobia: Dr Elkan has shown that they can have different phenotypes and require special detergent media to be seen. Finally, despite its use in California, I question Farnden and Robertson's recommendation of a tritium exchange to assay uptake hydroxylases: tritium uptake is more sensitive and theoretically sounder.

No doubt other criticisms could be made, but they all become trivial in the context of the excellence of the book as a whole. Dr Bergersen is to be congratulated on choosing authors with direct, practical experience of their subjects and ability to communicate that experience to the reader. The information in this book will have a very much longer half-life than that in most of the more theoretical books and the symposia on diazotrophy which are now being published with such regularity. This one is essential for any laboratory teaching or researching in this area and even experienced research workers will benefit from dipping into it. □

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T-cell controversies

Werner Haas

Regulatory T Lymphocytes. Edited by Benvenuto Pernis and Henry J. Vogel. Pp.449. ISBN 0-12-551860-9. (Academic: 1980.) \$47.50, £26.50.

THIS collection of papers and review articles about suppressor (T_s) and helper (T_h) T cells was written by a group of experts, mainly from the USA, who met in June 1979 at Columbia University. The majority of findings described are poorly understood and controversial; they will challenge the expert and confuse the non-expert.

Outstanding is the article by Sprent indicating that T_H interact only with B cells and macrophages expressing Ia antigens when encountered by the T cells in early differentiation. Confirmatory and contradictory experiments are discussed by Pierce and Kapp, by Mitchison and by Marrack *et al.* A more detailed analysis of regulatory T cells requires *in vitro* experiments. As yet, informative data are rare, but there are some studies in the right direction — selection in long-term cultures (Augustin *et al.*) and limiting dilution analysis of specific T_H (Zauderer *et al.*), and isolation of a T_S clone (Cantor and Gershon) are discussed here. Current experiments concerned with the serology and biochemistry of antigen-specific T cell receptors and factors are referred to in several articles and some details are described by Cramer and Krawinkel, Goodman *et al.* and Geha. Melchers and McDevitt present a clearly written, comprehensive review on the selective expression of Ia antigens by T cells. Functionally distinct human and murine T cell subsets identified by a variety of antisera and monoclonal antibodies feature in papers by Micklem *et al.*, Friedman *et al.* and Reinherz and Schlossmann; identification of subsets by red cells coated with different classes of antibodies is considered by Fauci and by

Moretta *et al.* An impressive amount of work, presented or reviewed by Fauci *et al.*, Siegal and Siegal, and Waldmann *et al.*, suggests that a variety of human diseases are associated with or caused by various forms of “disordered immunoregulation”.

There is general acceptance of Jerne's notion that antigen-specific receptors must be the primary target of any specific regulatory mechanism in the immune system. This experimentally demanding issue is considered in more detail in articles on idiotype-specific T_S by Bona and Paul and T_H by Eichmann and Simon, Ig dependent T_H by Janeway *et al.*, allotype suppression by Tokuhisa *et al.* and T cell mediation of resistance to graft versus host disease by Wilson *et al.* Finally, Herzenberg and colleagues demonstrate how to express complex ideas in clear drawings.

The book will be a disappointment to readers who expect more definitive reports on the various types of T_H and T_S , H-2 restriction of T_S , inducer cells and feedback inhibition, regulation of cytotoxic T cell responses or on the various types of T cell factors inducing lymphocyte proliferation and/or differentiation. □

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after-discharges in the same system. There is, however, a danger in generalizing from epileptics to normals since there must be something abnormal in the epileptic brain to cause the discharge in the first place.

In the absence of interesting new results to report, most of the contributors resort to the banal. It is no surprise that the brain waves produced in transcendental meditation resemble those in sleep or that they cannot be distinguished from those occurring when listening to soft music or during progressive relaxation. Nor is it particularly revealing that Italians asked to endure high levels of shock are sensible enough to call off the experiment sooner than do Yankee Protestants.

Much of the theorizing is at the same level of banality. C. E. Izard cites with approval W. Charlesworth's opinion, doubtless reached after years of wrestling with the problem, that “surprise is a misexpected object or event”, whilst Karl Pribram, in one of the few intelligible sentences in his chapter, enlightens the ignorant reader with the news that “Science pursues knowledge by observation and experiment”.

Most of the book's theorizing is so nonsensical that it is impossible to describe. Its level may be gauged by the following quotation from the chapter by R. J. Davidson, one of the editors:

The term ‘unconscious’ is here being used . . . (1) to refer to mental structures . . . which contour and transform both exogenous (from the environment) as well as endogenous (from within the person . . .) information, and (2) to refer to processing activity, action and/or information transmission in the nervous system which proceeds in the absence of conscious awareness . . . The first usage makes reference to internal mental (i.e. biocognitive) structures . . . The second usage is concerned with observable behaviour (either directly or indirectly) of which the subject is unaware.

One cannot evaluate Dr Davidson's claim to have uncovered two kinds of unconscious process, since he is clearly unable to put the difference into words: nor does he explain what is meant by “observable behaviour of which the subject is unaware”. In an equally silly chapter, Pribram argues that “feedforward” operations require consciousness: the reader who wants to know what feedforward is learns that it is “feedbacks joined into parallel processes”.

Much of the book — and all of its theoretical sections — is a farrago of pretentious rubbish; it is atrociously written, with the usual attempt to make commonplace ideas sound important by the introduction of neologisms. In short, the book is a disgrace to the publishers, the editors and the contributors. □

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Unconscious pretension in psychology

Stuart Sutherland

The Psychobiology of Consciousness. Edited by Julian M. Davidson and Richard J. Davidson. Pp.489. ISBN 0-306-40138-X. (Plenum: 1980.) \$32.50, £20.48.

ONE of the most significant changes in psychology over the past two decades is that consciousness has become respectable. Several separate developments have brought it back to fashion, including the realization that behaviourism can offer no explanation for many actions, the development of computer simulation as a method for modelling complex thought processes, the increasing interest in Western society in attaining “altered states of consciousness” either through psychotropic drugs or by meditative practices, and the discovery of many different transmitter substances that are involved in the regulation of mood. It is with the latter two themes that *The Psychobiology of Consciousness* is mainly concerned.

The field appears to be in such chaos that the attempt to think about it has reduced the book's 15 contributors to incoherence. Their strenuous efforts to resuscitate the shade of Titchener by recording and classifying states of consciousness, both normal and abnormal, have met with no success. The few interesting new findings

they present have little unity. Typical examples of such isolated discoveries are that since large quantities of marijuana usually have little effect on novices, new users of the drug have to learn how to become “stoned”, and that there is a tendency to look to the right (which may mean that the left hemisphere is being used) when thinking verbally, and to the left when working out spatial problems.

Perhaps of more significance are some recent studies of temporal lobe epilepsy. Synchronous spiking of nerve firing within the temporal lobe can be accompanied by feelings of ecstasy, religious awe and extreme kindness, and it is usually associated both with a lack of interest in sex and with the absence of any form of addiction. Treatment of the spike focus to eliminate the hypersynchrony often alters the epileptic's personality for the worse. His irritability increases, and he may become unhappy: he regains an interest in sex, but usually loses his religious preoccupations. Although A.J. Mandel does not go so far as to allege that St Paul had a seizure on the road to Damascus, he does argue that ecstasy is normally accompanied by hypersynchronous discharge in the limbic system excluding the amygdala and that the prolonged state of saintly kindness that ensues depends upon

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